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— SECTION C —

BOTANICAL SCIENCES

Contents

	Page
Cheminements Botaniques à travers Anticosti—*Jacques Rousseau*	225
Spike Density, Speltoidy, and Compactoidy in Hexaploid Wheat—*John Unrau, W. E. Smith, and R. C. McGinnis*	273
Tumor Induction in *Vicia faba* and Other Hosts by *Agrobacterium rubi* (Hildebrand) Starr and Weiss—*L. C. Coleman*	277
Phloem Necrosis of Potato Tubers in Relation to Leaf-roll-free *Myzus persicae* Sulc.—*T. R. Davidson*	283
Studies on the Parasitism and Variation of *Alternaria Raphani*—*R. G. Atkinson*	288
North American Species of *Chrysomyxa*—*D. B. O. Savile*	318
Increase in Girth of the Cambium in *Thuja occidentalis* L.—*Barbara E. Whalley*	331
The Elongation of Fusiform Cambial Cells in *Chamaecyparis*—*M. W. Bannan and Barbara E. Whalley*	341
Heterotrophic Nutrition of Seed Plants with Particular Reference to *Lemna minor* L.—*Paul R. Gorham*	356
Nuclear Conditions in Normal Stem Tissue of *Vicia faba*—*L. C. Coleman*	382

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CHEMINEMENTS BOTANIQUES A TRAVERS ANTICOSTI[1]

PAR JACQUES ROUSSEAU[2]

Abstract

Prior to 1940 the botanical surveys of Anticosti were restricted to the coast and seldom further inland than the lower parts of a few rivers. Following an initial visit to the center of the island in 1940, the author crossed the inland on foot from the mouth of the Vaureal to the mouth of Chaloupe creek in 1942. The present paper first describes the vegetation of the sector surveyed. Then follow a discussion on its economic importance in relation to wildlife, the description of new varieties, forms and hybrids, the complete list of species collected in the surveys of 1940 and 1942,—the main purpose of which was to supplement the documentation amassed by Prof. Marie-Victorin in preparation for his monograph on the Anticosti and Mingan flora,—and the author concludes with views on the phytogeography of Anticosti. Contrary to Prof. Marie-Victorin's hypothesis, no preglacial refuge exists in the center of the island and the presence of arctic and cordilleran relicts there may be postulated by the hypothesis of arctic and alpine outposts.

Introduction

Immense table calcaire aux strates horizontales, isolée dans le golfe Saint-Laurent, l'île Anticosti (fig. 1) se présente comme un coin bouchant l'entrée du fleuve, entre les roches apalachiennes plissées de la Gaspésie et la Côte-Nord, sur le rebord du plateau précambrien (62).

L'une des premières terres canadiennes à recevoir la visite d'Européens, Jacques Cartier (4, 54) y mouille, dans le voisinage de la pointe du Sud-ouest, le 25 juillet 1534, puis contourne l'île aux trois quarts entre le 27 juillet et le 1er août. Ile basse, simple ligne d'horizon. Ne percevant que la formation d'épinettes rabougries bordant la berge et les tourbières littorales, le navigateur remarque que "le tout desdites terres sont terres unyes, et les plus descouverte de boys que nous ayons veu et trouvé, avec belles praryes, et champaignes, vertes à merveilles".

Terreur des navigateurs, peuplée de légendes, manquant de mouillages, battue par le ressac qui enlise les carcasses des voiliers naufragés, l'île, après un essai d'habitation par l'hydrographe du roi, Louis Jolliet (8, 9), resta

[1]*Manuscrit reçu le 3 octobre 1949 et révisé, le 4 février, 1950.*

Contribution du Jardin botanique de Montréal, Montréal, Que.

[2]*L'auteur tient à remercier particulièrement tous ceux qui ont apporté leur collaboration au cours de ce travail, soit pour identifier une partie du matériel ou autrement, notamment MM. Marcel Raymond, M. L. Fernald, James Kucyniak, Ernest Rouleau, G. L. Stebbins, H. E. Graham, Raymond Gascon, Mmes Frances G. Weintraub, Béatrice Bergeron, Simone Constant, Germaine Crépeau.*

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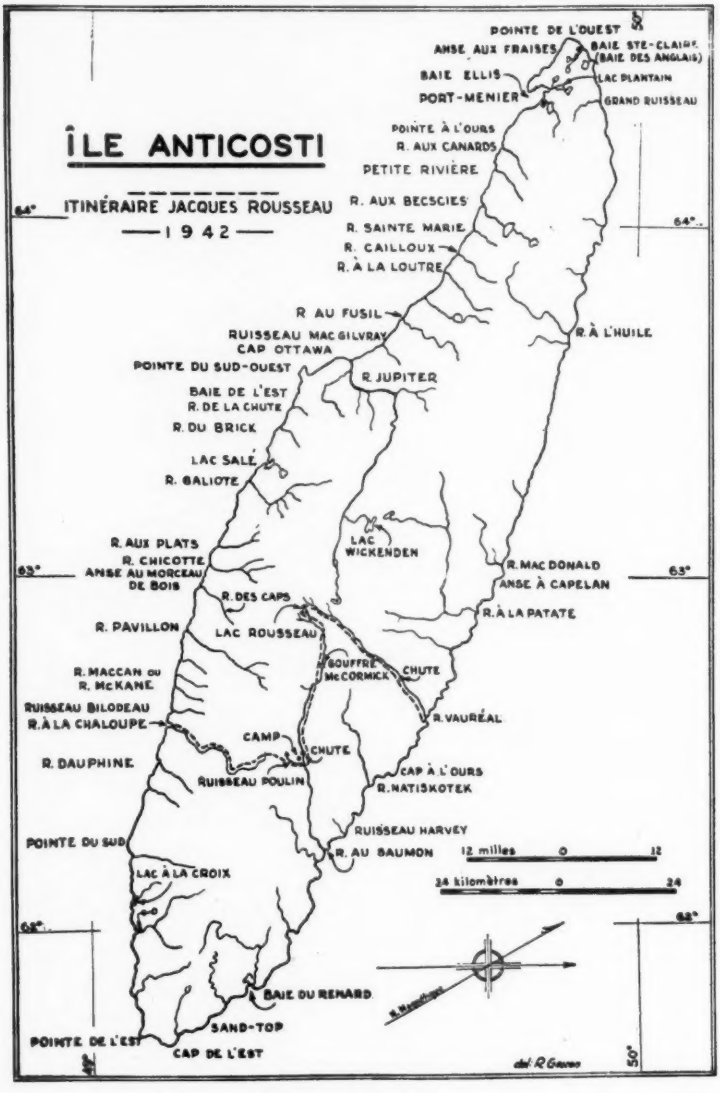

FIG. 1. *Carte de l'île.*

pratiquement fermée aux visiteurs jusqu'à la fin du siècle dernier. Pour longtemps, ce fut le repaire de rares chasseurs de la Côte-Nord et de "squatters", comme le légendaire Gamache, et le refuge transitoire de naufragers.*

Les botanistes eux-mêmes n'en entreprirent la visite qu'au milieu du siècle dernier. Après Pursh et Goldie, l'abbé Ferland (12, 13, 22), historien et naturaliste amateur, y fit quelques récoltes. Puis vinrent Richardson, Verrill, l'abbé Ovide Brunet, Macoun, Saint-Cyr, Schmitt (60), F. Marie-Victorin (35 à 46), F. Rolland-Germain et P. Louis-Marie, Adams et Jacques Rousseau.**

Ce sont les expéditions du F. Marie-Victorin et du F. Rolland-Germain, de 1925 à 1927, qui nous ont révélé le mieux les richesses de l'île. Le F. Marie-Victorin commençait, en 1944, à mettre au point un travail d'ensemble sur la flore de la Minganie et d'Anticosti, quand sa fin soudaine vint mettre un terme au projet. Toutefois, s'il n'a pas publié son étude définitive, quelques essais fragmentaires donnent néanmoins sa pensée sur les problèmes phytogéographiques soulevés par la présence d'une florule reliquale (36 à 46).

De la flore de l'intérieur de l'île, nous ne savions rien. C'était donc pour contribuer aux recherches du F. Marie-Victorin et mettre à l'épreuve certaines de ses hypothèses phytogéographiques, avant qu'il ne s'attaque à l'étude définitive, que l'auteur se rendit en avion au centre de l'île, en 1940 (55), et la traversa à pied, du nord au sud, dans la partie la plus prometteuse, en 1942. Ce dernier voyage fait l'objet du présent mémoire. Les aspects ethnobotaniques des voyages de 1940 et 1942 ont été traités toutefois dans un autre mémoire (56).

Ayant eu l'occasion, en 1940, de parcourir à pied la grève depuis la rivière Sainte-Marie jusqu'à quelques milles à l'est de la rivière à la Loutre, l'auteur avait pu visiter l'embouchure de rivières ou ruisseaux inexplorés jusque là parce qu'en beaucoup d'endroits, il est très difficile d'aborder en chaloupe pontée. Cette marche avait permis la découverte d'éléments intéressants. De là semblable excursion, en 1942, depuis le crique à la Chaloupe jusqu'à la rivière Jupiter.

Traversée de l'Île

Vers la Vauréal

Une envolée au lac Wickenden, en 1940, permettant une herborisation de quelques heures, avait fourni les premiers renseignements botaniques sur le centre de l'île. Toutefois, pour toutes fins pratiques, l'intérieur demeurait inconnu. Pour remédier à la pauvreté de nos connaissances, rien ne valait une traversée à pieds. L'itinéraire choisi fut le suivant: monter à la source de la Vauréal, rallier la source de la rivière au Saumon, descendre cette rivière jusqu'à une dizaine de milles de la mer, traverser à la source de la rivière à

** Les squatters sont des personnes qui s'établissent sur des terres sur lesquelles elles n'ont aucun droit de propriété ni de résidence. Sur les différents aspects de l'histoire d'Anticosti nécessaires pour une meilleure connaissance de l'exploration de l'Ile, voir bibliographie (3, 11, 13, 26, 28, 35, 49, 60, 64).*

*** Pour la bibliographie des botanistes ayant visité Anticosti aux dix-neuvième et vingtième ièc1es, voit la prochaine étude de Marcel Raymond sur les Carex d'Anticosti.*

la Chaloupe et suivre cette dernière jusqu'à la mer. L'île Anticosti mesure 135 milles de long par 35 milles dans la partie la plus large (soit 217 kilomètres par 56). Cette traversée en zigzag, évaluée à 90 milles environ, est la première encore faite à pieds, si l'on excepte des courses en raquettes aux deux extrémités.

Le 7 juillet 1942, départ en chaloupe pontée de Port-Menier pour l'embouchure de la Vauréal, en compagnie de Charley McCormick, Vilbon Collard et Fernand Noël qui servirent de guides et porteurs. M. H. E. Graham, administrateur de l'île pour la compagnie propriétaire, la Consolidated Paper Corporation, qui a tant contribué à faciliter le voyage, accompagne le groupe jusqu'à l'embouchure de la Vauréal, d'où aura lieu le départ le lendemain matin.

Vers la chute de la Vauréal

De la mer à la chute, il y a près de sept milles. Il faut marcher habituellement sur le gravier de la rivière, traverser à chaque courbe, l'eau jusqu'aux genoux. Si le hasard nous conduit dans la forêt, le parterre moussu est moins dur pour le pied, mais il faut par contre enjamber les arbres renversés, dans une atmosphère lourde de moustiques. Rares sont les personnes qui se sont rendues à cette chute (fig. 2), la plus belle de la province. D'une falaise élevée, la rivière se précipite dans une grande tranchée calcaire, d'un mille de long (fig. 3). Jusqu'à ce canyon, la vallée est large et le fond plat est couvert de grandes platières exondées au cours de l'été, que les Anticostiens nomment des "battures". Il y croît en certains points quelques arbres et arbustes:

Fraxinus nigra	*Larix laricina*
Juniperus communis var. *depressa*	*Picea mariana*
Juniperus horizontalis	*Pinus Strobus*

Ces platières toutefois sont surtout couvertes d'une formation gazonnante pouvant supporter plus facilement que les arbres précédents une submersion printanière, et comprenant notamment:

Allium Schoenoprasum var. *laurentianum*	*Carex Crawei*
Antennaria gaspensis	*Carex lepidocarpa*
Antennaria spathulata	*Dryas Drummondii*
Carex aquatilis	*Sanicula marilandica*
Carex Buxbaumii	var. *borealis*

C'est l'époque où les *Dryas* déploient leurs plumules. Il est étrange de voir cette espèce arctique-alpine dans un habitat submergé au printemps, avec des plantes d'habitats marécageux. Ce que les *Dryas* cherchent sur les platières,—avec d'autres espèces arctiques-alpines,—ce n'est pas la submersion mais l'absence de compétiteurs forestiers qui viendraient interposer l'écran de leur feuillage. C'est d'ailleurs cette exigence de la lumière vive qui les rend propices aux sommets des montagnes et à la toundra arctique. Ces espèces, au surplus, ont une saison de végétation très courte; aussi la submersion printanière, avant le réveil de la végétation, ne les incommode aucunement, d'autant plus que leurs racines les ancrent solidement au sol.

FIG. 2. *Chute de la rivière Vauréal.* FIG. 3. *Le canyon au pied de la chute de la Vauréal.* FIG. 4. *Petites platières de la Vauréal, un mille en amont de la chute. Cette photo est typique de l'aspect habituel de la Vauréal supérieure.*
FIG. 5. *Sur la Vauréal supérieure, un secteur de la forêt a été détruit par l'établissement d'un barrage de castors.*

A plusieurs reprises, le F. Marie-Victorin et le F. Rolland-Germain ont visité les platières à l'embouchure de la Vauréal. Le F. Rolland, d'autre part, s'est rendu une fois au pied de la chute. Aussi nous ne nous attardons pas pour aller camper au-dessus, en territoire inconnu.

C'est du haut de la chute qu'il faut voir le grand canyon blanc, tortueux, aux strates horizontales (fig. 3). Au bas, sur les talus ayant la pente d'éboulement de la matière libre, se donnent rendez-vous des plantes rares capables de s'aggripper aux matériaux lâches par un puissant système radiculaire. C'est là que pousse l'*Aster vaurealis* Rousseau (55), l'un des *Aster* les plus étranges, décrit d'après des spécimens rapportés par le F. Rolland-Germain.

Cours supérieur de la Vauréal

Pour accéder au sommet de la chute, il faut s'enfoncer dans la forêt, à l'est de la rivière, avant l'entrée du canyon. Une heure et demie de portage sur une pente abrupte. En amont de la chute, la rivière a dorénavant un tout autre aspect (fig. 4). Absentes, les immenses platières graveleuses, absentes aussi, la plupart des espèces arctiques-alpines qui fréquentent d'habitude l'embouchure des rivières d'Anticosti. A part trois petits affleurements calcaires, la forêt ou la tourbière se rendent le plus souvent jusqu'à la rivière, qui coule sur des dalles calcaires. C'est là qu'il faut marcher généralement, heureux de rencontrer d'espace en espace des dépressions vaseuses couvertes de *Carex* broutés par les cerfs ou de minuscules plages graveleuses qui ne ressemblent plus par la flore aux platières de l'embouchure. La rivière est généralement étroite, peu profonde et se traverse facilement à pieds.

Le premier affleurement calcaire au-dessus de la chute héberge:

Achillea lanulosa
Allium Schoenoprasum var. *laurentianum* et forme albino*
Anemone hudsoniana
Anemone parviflora
Antennaria gaspensis
Arnica chionopappa
Campanula rotundifolia
Carex eburnea
Carex flava
×*Carex Pieperiana*
Castilleja septentrionalis
Cystopteris bulbifera
Festuca rubra
Glyceria striata
Hieracium groenlandicum
Juncus alpinus
Linnaea borealis var. *americana*
Poa alpina
Primula mistassinica
Rosa johannensis
Saxifraga aizoides
Scirpus cespitosus var. *callosus*
Senecio gaspensis
Solidago hispida
Tofieldia glutinosa
Trisetum spicatum var. *molle*
Zigadenus glaucus

Cette liste comprend la flore à peu près complète des falaises calcaires verticales. Il s'y trouve donc encore des espèces de type arctique-alpin, mais beaucoup moins nombreuses que sur les platières de l'embouchure.

Au deuxième affleurement calcaire,—plutôt argilo-calcaire,—trois milles plus haut, il ne reste d'arctique-alpin que l'*Arnica chionopappa* poussant avec *Salix*

* *Forme décrite plus loin.*

Bebbiana, *Juniperus communis* var. *depressa*, *Polygonum viviparum* et *Cladonia* sp. (groupe du *Cladonia alpestris*). Le troisième, dix milles en amont de la chute, ne ressemble plus par sa flore aux rochers calcaires avoisinant la mer. Il y croît en effet:

Antennaria canadensis
Apocynum androsaemifolium
Habenaria dilatata
Petasites palmatus
Populus tremuloides var. *rhomboidea*

Le *Populus tremuloides* est rare sur l'île. Les castors auraient eu raison des formations qui y abondaient. Manquant de tremble, le castor s'est tourné vers d'autres arbres et arbustes; les conifères, même, entrent désormais dans leur menu. Avec un tel régime, l'animal est presque disparu de l'île. Notons aussi que des digues de castors barraient autrefois la Vauréal, comme en témoignent de grandes étendues où les arbres sont morts par suite de l'inondation (fig. 5).

A part les affleurements rocheux, les seuls habitats susceptibles d'héberger une flore arctique-alpine sur le cours supérieur de la rivière sont les petites platières et la berge graveleuse.

L'un des meilleurs exemples de ces petites platières graveleuses se trouve à environ un mille en amont de la chute (fig. 4). Il y croît notamment:

Anaphalis margaritacea
Caltha palustris
Carex angustior
Carex aquatilis
Carex Buxbaumii
Carex lepidocarpa
Carex limosa
*Carex viridula**
Glyceria striata
Iris versicolor
Muhlenbergia Richardsonis
Pyrola asarifolia var. *incarnata*
Ranunculus reptans
*Viola affinis***

C'est donc surtout une formation de *Carex* et de Graminées. A part quelques arbustes forestiers, ces *Carex* et Graminées constituent la principale nourriture des chevreuils sur l'île. (Chaque fois qu'il sera question de "chevreuil" dans ce texte, entendons par là le cerf de Virginie auquel on a transposé chez les Canadiens français le nom d'un cervidé européen.)

Ces platières graveleuses sont plutôt rares sur la Vauréal supérieure. Le plus souvent, la forêt est séparée de la grève par une simple bordure de végétation arbustive. Parfois, la tourbière se rend jusqu'à la rivière et l'on trouve alors associés, à peine au-dessus du niveau actuel de la rivière:

Andromeda glaucophylla
Carex sterilis
Drosera rotundifolia
Eriophorum viridicarinatum
Ledum groenlandicum
Menyanthes trifoliata
Rubus Chamaemorus
Sarracenia purpurea
Thalictrum alpinum
Triglochin maritima
Utricularia intermedia
Vaccinium Vitis-Idaea var. *minus*
etc.

* *Forme nouvelle décrite dans une autre étude par Marcel Raymond.*
** *Nouvelle variété décrite plus loin.*

Plus fréquemment, le rivage bas est recouvert d'une véritable formation de prairie, comprenant nombre d'espèces des petites platières précédemment décrites, et surtout de grandes herbes, telles que *Carex Buxbaumii*, *Muhlenbergia Richardsonis*, entremêlés d'*Iris versicolor*. Il n'a pas été possible de constater si les chevreuils consommaient l'*Iris*, toxique pour l'homme, mais les deux autres, sur cette rivière, semblent la base de leur alimentation.

Source de la rivière

Partis de la mer le 8 juillet au matin, le 9 nous campons en face du sentier de la source de la Jupiter. Un mille et demi plus loin que le sentier, soit à 23 milles de la mer approximativement et à une couple de milles de la source de la Vauréal, nous abandonnons cette rivière pour la source de la rivière au Saumon. L'obligation de tout porter sur le dos, et de différer le séchage des spécimens jusqu'à l'arrivée à la côte, impose des restrictions aux récoltes. La végétation est très pauvre d'ailleurs. Dans le voisinage de la source, la Vauréal n'offre aucun affleurement rocheux, mais plutôt une zone de tourbières. La berge étroite et basse,—car la forêt est à peine au-dessus du lit de la rivière, —est tantôt graveleuse, tantôt vaseuse ou légèrement tourbeuse. Le plus souvent, elle est bordée par un cordon arbustif où abondent *Betula pumila*, *Myrica Gale* et parfois *Viburnum edule*, le pimbina de l'est, mais extrêmement rare parce que constamment brouté par les chevreuils et les lièvres. La flore des rives à la source de la Vauréal comprend surtout:

Allium Schoenoprasum var. *laurentianum* et l'albino
Anemone parviflora
Arctostaphylos Uva-Ursi var. *coactilis*
Abies balsamea f. *hudsonia*
Betula pumila
Campanula rotundifolia
Carex Buxbaumii
Carex Crawei
Danthonia sp.
Equisetum arvense var. *boreale*
Erigeron hyssopifolius
Galium labradoricum
Larix laricina f. *depressa*
Muhlenbergia Richardsonis
Myrica Gale
Petasites palmatus
Picea mariana f. *squamea*
Pinguicula vulgaris
Polygonum viviparum
Prunella vulgaris
Primula mistassinica
Rhamnus alnifolia
Sanguisorba canadensis
Schizachne striata
Scirpus cespitosus var. *callosus*
Senecio aureus var. *aquilonius*
Senecio gaspensis
Sisyrinchium montanum
Taraxacum lapponicum
Thalictrum alpinum
Tofieldia glutinosa
Viburnum edule
Viola affinis n. var.
Zigadenus glaucus

De la source de la Vauréal à la source de la rivière au Saumon

Toutes les nuits, il fait froid et, la nuit dernière, le mercure est descendu au voisinage de 0° C. Le jour, par contre, la température très élevée rend la marche pénible, surtout dans la forêt et les tourbières. La région entre la Vauréal et la rivière au Saumon n'a jamais été explorée. Cest donc un peu

au hasard que nous cherchons la source de la rivière au Saumon. Personne, en effet, ne sait jusqu'où exactement se rend cette rivière. En laissant la Vauréal, la forêt est claire, de type subalpin, et après avoir trébuché quelque temps sur les "petits Saint-Michel" (les épinettes rabougries), l'on aborde une grande tourbière,—une "plaine", suivant l'expression de l'île,—où la marche est pénible. Tourbière surélevée, dominée par un lac d'un mille de long, encore inconnu, que les guides dédient à l'auteur et situé à la source même de la rivière au Saumon. La traversée directe à la rivière au Saumon aurait été de deux milles à travers une forêt parsemée d'épinettes rabougries. Bien que la marche sur un matelas de sphaigne où l'on enfonce est pénible, il semble que le trajet de quatre milles, parcouru en trois heures, est encore plus aisé que la route directe.

Cette grande tourbière renferme surtout les éléments suivants, dont le *Betula Michauxii* et le *Carex Michauxiana* constituent les premières récoltes sur l'île:

Andromeda glaucophylla
Arethusa bulbosa
Betula Michauxii
Betula pumila
Carex Michauxiana
Carex microglochin
Chamaedaphne calyculata
Drosera anglica
Drosera intermedia
Drosera rotundifolia
Empetrum nigrum
Equisetum limosum
Eriophorum Chamissonis
Eriophorum viridicarinatum
Habenaria dilatata
Larix laricina f. *depressa*
Ledum groenlandicum
Menyanthes trifoliata
Myrica Gale
Nuphar variegatum
Pinguicula vulgaris
Rubus Chamaemorus
Salix candida
Sanguisorba canadensis
Sarracenia purpurea
Scheuchzeria palustris var. *americana*
Scirpus hudsonianus
Tofieldia glutinosa
Vaccinium Oxycoccos

De la source de la rivière au Saumon au gouffre McCormick

La rivière au Saumon débute comme un petit ruisseau issu du lac Rousseau. En un mille, il se transforme en une rivière coulant sur des dalles calcaires farcies de coraux fossiles en cornets. L'espèce la plus commune à la tête de la rivière est le *Zaphrentes hannah* Twenhofel, de la formation Becscie ou de la formation Gun river. A la source de la Vauréal, par contre, se trouvaient des *Streptelasma selectum* Billings, de la formation Ellis bay. Les spécimens ont été identifiés par W. H. Twenhofel. Les fossiles sont à demi détachés; aussi on a l'impression de marcher sur une râpe. En moins de deux jours, ils ont raison des chaussures.

La rivière au Saumon ressemble beaucoup à la Vauréal supérieure, mais les dalles calcaires tapissent le fond de la rivière sur un plus grand parcours. Une automobile pourrait voyager sur le lit de la rivière sans trop de difficultés sur la plus grande partie du cours.

Dans la partie supérieure, un minuscule barrage, fait de bois fraîchement coupé, témoigne de la présence d'une famille de castors; mais il n'y a aucun tremble dans les parages.

Les habitats sont les mêmes que sur la Vauréal supérieure: berge basse aux strates calcaires horizontales, ou plage graveleuse couverte d'une végétation gazonnante comme sur la Vauréal, ou cordon littoral d'arbustes séparant la forêt d'épinettes du cours de l'eau.

La rivière au Saumon, comme la Vauréal, renferme des potamots, *Potamogeton gramineus* et *P. filiformis* var. *borealis*, surtout le premier. Parfois aussi *Nuphar variegatum*, mais jamais abondant. Sur la Vauréal, les potamots observés n'avaient ni fleurs ni fruits.

Sur la berge argilo-calcaire, près de la source, voisinent le *Solidago hispida* et l'*Hieracium groenlandicum*. Quant aux platières calcaires, depuis la source jusqu'au gouffre, elles hébergent une florule ressemblant beaucoup à quelques éléments près, à celle de la Vauréal supérieure. Il y pousse:

Allium Schoenoprasum var. *laurentianum*
Anemone multifida var. *hudsoniana*
Anemone parviflora
Antennaria neodioica var. *chlorophylla*
Campanula rotundifolia
Carex Buxbaumii
Carex Crawei
Carex lepidocarpa
Castilleja septentrionalis
Danthonia sp.
Erigeron hyssopifolius
Galium labradoricum
Glyceria striata
Iris versicolor typique et n. var.
Juncus alpinus var. *fuscescens*
Myrica Gale
Petasites palmatus
Pinguicula vulgaris
Polygonum viviparum
Potentilla fruticosa
Primula mistassinica
Prunella vulgaris
Senecio aureus var. *aquilonius*
Senecio gaspensis
Sisyrinchium montanum
Taraxacum lapponicum
Thalictrum alpinum
Triglochin palustris n.f.
Vaccinium canadense
Viburnum edule
Viola affinis n. var.
Zigadenus glaucus

Gouffre McCormick

A six milles de la source, la rivière s'enfonce dans le sol par une crevasse coupant tout le lit sur une longueur de cent pieds et une profondeur de vingt-cinq pieds (fig. 6). L'eau s'y engouffre et ne retourne que graduellement dans le lit de la rivière. Deux milles plus loin, la rivière semble devenue normale. Dans l'intervalle, l'ancien lit du cours d'eau ressemble à des montagnes russes par ses ondulations. Les plantes qui ont colonisé l'ancien lit sont les espèces ordinaires des grèves graveleuses de la Vauréal supérieure et de la source de la rivière au Saumon. De jeunes épinettes noires (*Picea mariana*) de trois à cinq ans au plus témoignent de l'âge récent du gouffre. Un arpenteur, passé ici vers 1930, n'en fait aucunement mention. Le guide McCormick, descendant la rivière en canot en 1935, remarqua brusquement le lit à sec à quelques centaines de pieds en avant. Intrigué, il laisse son canot sur la grève, continue

à pied et découvre ce gouffre auquel j'attache son nom. Sans aucun doute, nous assistons là actuellement au creusement d'un petit canyon.

La rivière étant à sec, en aval du gouffre, on peut examiner à loisir de vieilles chaussées de castor. Elles étaient formées non seulement de branchages, mais de troncs d'arbres d'un pied de diamètre, de cailloux et de terre.

Du gouffre McCormick au canyon de la rivière au Saumon

Ayant campé trois milles en aval du gouffre, nous parcourons le 11 juillet les vingt-cinq milles qui nous séparent du camp de la rivière au Saumon, situé à une dizaine de milles de la mer, en aval de la chute, à l'entrée du canyon. Au début de la journée, marche pénible dans les grandes herbes mouillées cachant des troncs d'arbres renversés et des trous de deux pieds de profond, comme taillés à l'emporte-pièce. Ces prairies sont des formations de *Carex* presque pures. Comme sur la Vauréal, il ne se passe pas cinq minutes sans rencontre de chevreuils qui broutent. Les principales espèces de l'association sont:

Agropyron trachycaulum
Carex angustior
Carex aquatilis
Carex Buxbaumii
Carex castanea
Carex flava
Carex leptalea
Carex rostrata var. *ambigens*
Eriophorum viridicarinatum
Galium labradoricum
Iris versicolor
Myrica Gale
Poa saltuensis
Senecio aureus
Sanguisorba canadensis
Thalictrum polygamum
Viburnum edule

Dans la zone bordée de prairies, la rivière coule lentement sur un lit vaseux où les potamots,—notamment *Potamogeton gramineus* et *P. panormitanus*,—s'implantent facilement.

Le secteur des prairies passé, la rivière roule sur des dalles calcaires, étroitement serrées par la forêt. La cueillette n'offre alors rien d'inusité. A 25 milles de la mer apparaissent définitivement les corniches calcaires où se rencontrent en partie les éléments de la chute Vauréal. Il y croît:

Allium Schoenoprasum var. *laurentianum* et l'albino
Anemone virginiana
Antennaria gaspensis
Arnica chionopappa
Carex lepidocarpa
Danthonia sp.
Deschampsia caespitosa
Festuca prolifera var. *lasiolepis*
Hedysarum alpinum var. *americanum*
Scirpus rubrotinctus
Selaginella selaginoides
Solidago hispida
Taraxacum lapponicum

A cette saison, les grandes ciboulettes (*Allium Schoenoprasum*), dispersées, égayent de leurs fleurs roses le bord des cours d'eau et parfument toutes les rivières d'Anticosti.

Camp de la chute au Saumon

Après une marche de douze heures et quarante-cinq minutes, nous sommes arrivés, exténués, au camp de la chute au Saumon. En aval, la rivière coule

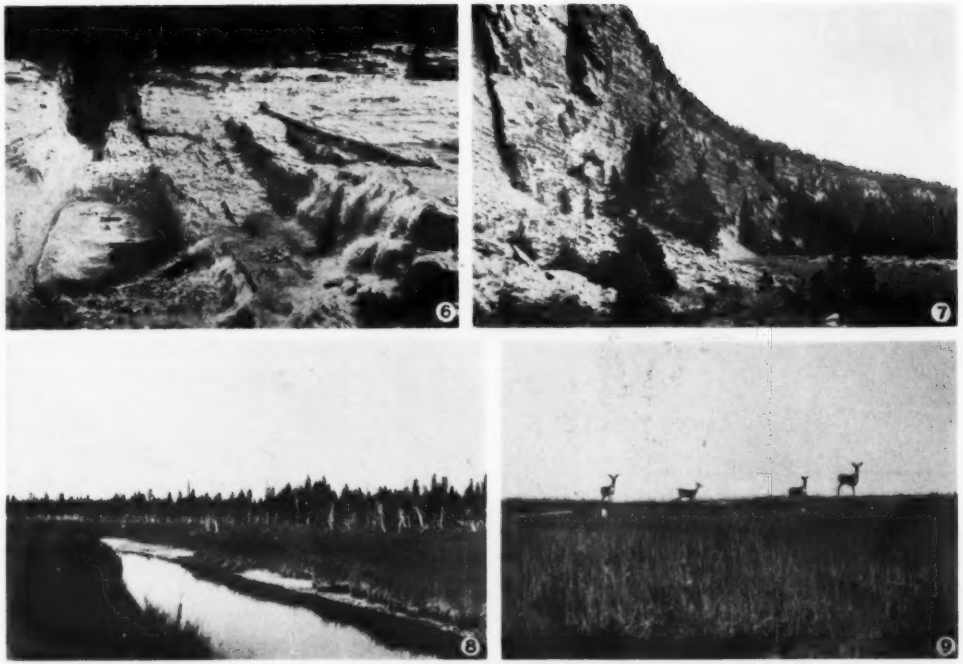

FIG. 6. *Le gouffre McCormick, dans la partie supérieure de la rivière au Saumon.* FIG. 7. *Les falaises de la rivière au Saumon à une dizaine de milles de la mer.* FIG. 8. *Rivière à la Chaloupe, près de la source.* FIG. 9. *Cerfs de Virginie paissant dans des prairies naturelles au bord de la mer.*

FIG. 10. *Allium Schoenoprasum var. laurentianum, la ciboulette sauvage d'Anticosti. Iris Hookeri; au centre, l'hybride, Iris Sancti-Cyri.* FIG. 12. *Gentiana nesophila.*

FIG. 11. *A gauche, Iris versicolor; à droite,*

dans un canyon, entre de hautes falaises calcaires, bordées à la base par un talus rocheux (fig. 7). Entre le talus et la rivière, la platière graveleuse héberge:

Deschampsia caespitosa
Dryas Drummondii
Prunella vulgaris var. *lanceolata*

Comme on a pu le constater sur la Vauréal, la rivière au Saumon et d'autres cours d'eau, le *Dryas Drummondii* n'habite que la partie inférieure des rivières.

Sur le talus de matériaux lâches ayant la pente d'éboulement de la matière libre, au pied de la falaise, aucun de ces éléments rares comme l'*Astragalus scrupulicola* et l'*Oxytropis gaspensis* des habitats analogues du Mont Saint-Pierre, en Gaspésie. Il y croît:

Anemone multifida var. *hudsoniana*
Antennaria gaspensis
Arctostaphylos Uva-ursi var. *coactilis*
Arnica chionopappa
Asplenium viride
Danthonia sp.
Diervilla lonicera
Draba laurentiana
Epilobium angustifolium var. *macrophyllum*
Juniperus horizontalis
Poa laxa
Prunus depressa
Pyrola virens
Salix Bebbiana
Senecio pauperculus var. *Balsamitae*
Solidago hispida
Taraxacum ceratophorum
Trisetum spicatum var. *pilosiglume*

De la rivière au Saumon à la source de la rivière à la Chaloupe

Après avoir herborisé tout le dimanche autour du camp de la rivière au Saumon, départ vers le sud le 13 juillet, à cinq heures du matin, suivant d'abord le ruisseau Poulin qui se déverse près du camp, sur un talus à pente d'éboulement de la matière libre. Cette pente de déjection est couverte de *Dryas Drummondii*. Le lit du ruisseau où ne coule qu'un mince filet, est tapissé de *Senecio aureus*. La berge abrupte de ce canal est couverte de *Petasites palmatus* et de *Thalictrum alpinum*. A un endroit, le ruisseau s'enfonce dans un gouffre d'une dizaine de pieds de profond et continue sous terre.

Le ruisseau vient d'une table calcaire irrégulièrement gazonnée: ce qui, à première vue, semble un affleurement alpin n'est qu'un lit évasé. Cette formation gazonnante comprend surtout deux graminées: *Danthonia* sp. et *Poa alpina*, auxquelles s'ajoutent quelques éléments disséminés, *Antennaria neodioica* var. *chlorophylla*, *Orobanche uniflora* var. *terrae-novae*, *Senecio gaspensis* var. *firmifolius*.

Au-delà du réservoir d'alimentation du ruisseau, une forêt claire de type subalpin avec sous-bois de *Pteridium aquilinum* var. *lanuginosum*, une variété cordillérienne, présente également sur les serpentines de Black Lake et en Gaspésie. Le bois subalpin et les calcaires superficiels à la source du ruisseau auraient pu laisser espérer quelque monticule alpin à la hauteur des terres mais il en est ici comme à la ligne de partage des eaux entre la Vauréal, la Jupiter et la rivière au Saumon. En effet, il n'y a que des tourbières surélevées

servant sans doute de réservoir commun aux cours d'eau des deux côtés de l'île. En survolant l'île depuis Port-Menier jusqu'au lac Wickenden, en 1940, la région s'était révélée une table uniforme, sensiblement de même hauteur au centre et au nord, couverte partout de lacs, de tourbières, et de bois d'épinettes. Le voyage de 1942, à son tour, éliminait tout espoir de trouver des élévations rocheuses au point d'où partent les principales rivières de l'île. Depuis, un vol à 8,000 pieds d'altitude, en revenant de l'Ungava, confirma cette observation. Nous pouvions voir les deux rives de l'île à la fois. La parfaite visibilité permettait de distinguer les moindres détails du terrain et même de reconnaître le chemin parcouru à pied, cinq ans plus tôt. Pas la moindre élévation rocheuse qui aurait pu servir de refuge à des espèces arctiques-alpines conformément à l'hypothèse du F. Marie-Victorin (44, 46).

La tourbière, entre la rivière au Saumon et le crique de la Chaloupe, renferme sensiblement les mêmes éléments que la tourbière entre la Vauréal et la rivière au Saumon. Il y croît:

Arethusa bulbosa
Betula Michauxii
Betula pumila
Carex limosa
Drosera anglica
Eriophorum angustifolium
Eriophorum spissum
Habenaria dilatata
Primula mistassinica
Scheuchzeria palustris var. *americana*
Scirpus hudsonianus

Cours supérieur du crique à la Chaloupe

Cinq milles après avoir laissé la rivière au Saumon, nous arrivons au crique à la Chaloupe, dont le cours est beaucoup plus étendu qu'on ne le croyait. Nous sommes à vingt-et-un milles de la mer et la source semble se trouver une couple de milles à l'ouest. La rivière coule d'abord dans une tourbière (fig. 8), à sous-sol graveleux. Les eaux lentes permettent la croissance de *Potamogeton filiformis* var. *borealis* et *Potamogeton gramineus*.

Cinq milles plus bas que le point d'arrivée, la rive graveleuse s'étend graduellement. La rivière alors ressemble beaucoup à la Vauréal supérieure et en héberge plusieurs éléments. Il y croît notamment *Allium Schoenoprasum* var. *laurentianum*, *Taraxacum lapponicum*, etc. Dans le bois de *Picea mariana*, ici et là, *Arethusa bulbosa*.

A treize milles du point où nous avons abordé la rivière à la Chaloupe, soit à huit milles de la mer, les platières graveleuses ressemblent à celles du cours inférieur de la rivière au Saumon, de la Jupiter, de la Vauréal, de la Chicotte et des autres rivières à platières d'Anticosti. Les *Dryas* y abondent, mais par contre les entités les plus rares, *Braya*, *Lesquerella*, etc., en sont absentes. On y trouve:

Allium Schoenoprasum var. *laurentianum* et l'albino
Antennaria spathulata
Arctostaphylos Uva-ursi var. *coactilis*
Dryas Drummondii
Eriophorum viridicarinatum
Pinguicula vulgaris
Polygonum viviparum
Salix pellita f. *psila*

Campanula rotundifolia	*Senecio gaspensis*
×*Carex Pieperiana*	*Sisyrinchium montanum* var. *crebrum*
Danthonia sp.	*Thalictrum alpinum*
Deschampsia caespitosa	*Zigadenus glaucus*

Près de l'embouchure de la rivière, dans un bois de seconde venue, une forme à feuilles luisantes du *Vaccinium uliginosum* var. *pubescens* croît en compagnie du *Vaccinium myrtilloides*.

Partis de l'embouchure de la Vauréal le 8 juillet au matin, nous arrivons à l'embouchure du crique à la Chaloupe le 14 juillet, à 9h. 30 du matin, ayant couché la veille à huit milles de la mer. Le voyage a donc duré six jours dont une journée d'arrêt. Comme il faisait froid toutes les nuits, les plantes se sont bien conservées dans les cartables, simplement enveloppés dans des toiles imperméables. A l'embouchure, il fallut consacrer près d'une journée au séchage.

Végétation et vie animale

Le gibier d'importance économique, dépendant directement de la vie végétale de l'Ile, comprend le lièvre, l'orignal, le castor et le cerf. Le premier, dont le court cycle d'abondance et d'absence est assez mal expliqué, trouve la nourriture qu'il lui faut et les ennemis susceptibles d'empêcher un pullulement exagéré.

L'orignal est extrêmement rare. Introduit par le sénateur Menier, vers 1900, il n'a jamais eu un grand succès depuis. Cela s'explique, car les grands nénufars jaunes sont peu abondants. D'autre part, la végétation arbustive, seule nourriture d'hiver, est trop systématiquement broutée par les cerfs de Virginie.

Le castor, introduit également par Menier, a pullulé déjà, mais il est en voie de disparition. La destruction des trembles (*Populus tremuloides*) à l'époque où l'animal abondait en est probablement la cause. Les rares familles qui subsistent semblent condamnées à consommer des conifères et des petits bouleaux nains. L'erreur des propriétaires a été de laisser se multiplier ces rongeurs, plus que ne le permettait la ressource en tremble, leur principale nourriture. Quand la population eût atteint son faîte, il fallut de quelques années seulement pour que la nourriture fasse défaut. Il serait illusoire d'interdire définitivement la chasse au castor dans les régions sauvages de la province. Sans doute, on pourrait, avec succès, interdire la chasse pour quelques années, dans un secteur, mais dès que le nombre d'individus aura dépassé un certain palier, l'ouverture de la chasse deviendra nécessaire. Autrement, la consommation des animaux dépassant le taux de récupération de la végétation, la famine décimera irrémédiablement la population. Malgré la rareté du castor sur Anticosti, l'interdiction de la chasse n'aurait aucune utilité. En effet, il suffit actuellement de quelques familles seulement pour mettre en échec la pousse des trembles. Si l'on envisage le problème sur un angle économique, il vaut mieux que le castor disparaisse de l'Ile pour permettre au tremble de se rétablir. Après quoi, il suffira d'introduire l'animal

de nouveau et, après quelques années, organiser une chasse méthodique empêchant la surpopulation. Une bonne partie d'Anticosti pourrait être ainsi transformée, de façon profitable, en ferme de castors. D'ailleurs, la coupe intensive des épinettes, depuis la guerre, favorisera la reprise du tremble.

Le cerf de Virginie, nommé chevreuil au Canada, faisait presque défaut lorsque Menier acquit l'Ile à la fin du siècle dernier. Repaire de braconniers, depuis des générations, l'Ile avait vu sa population animale s'anémier. Menier introduisit notamment le castor, l'orignal et le cerf de Virginie. Désormais protégés, les cerfs se multiplièrent beaucoup (fig. 9), bien que constituant la nourriture habituelle des gardiens de rivières et des amateurs de pêche au saumon fréquentant l'Ile l'été. Le jour du départ de l'expédition pour la Vauréal en 1942, à quatre heures du matin, sur le trajet d'une douzaine de milles séparant Port-Menier de la baie Sainte-Claire, il a défilé en une demi-heure quatre-vingt-seize têtes, dont un spécimen remarquable ayant la moitié du corps blanc. Dans cette partie habitée de l'Ile, on ne chasse pas le chevreuil: il se sent donc en sécurité et pénètre même dans le village. D'autre part, Menier ayant fait là des essais de culture, les terres abandonnées sont devenues des prairies naturelles favorables au cerf. Le long des rivières en 1940 et 1942, on ne pouvait marcher dix minutes sans rencontrer des chevreuils, en groupes de cinq ou six parfois. Sans doute, des grandes parties de l'Ile, trop marécageuses, ne conviennent pas à cet animal, mais les formations gazonnantes de *Carex* et de graminées, riches et abondantes, expliquent leur pullulement. L'évaluation de la population des cerfs, lors de la traversée de 1942 (tenant compte de tous les facteurs défavorables), s'est élevée aux environs de 15,000 têtes. C'est réellement trop pour l'Ile. Des prairies gazonantes, littéralement fauchées, ne renfermaient aucun spécimen assez complet pour un herbier. Ailleurs, des chevreuils émaciés témoignaient de la décroissance de la nourriture. Les arbustes comestibles étant, pour la plupart, broutés à une couple de pieds du sol, une neige abondante pourrrait, en un seul hiver, compromettre la vie des cervidés de l'Ile. Il semblait donc désirable d'en réduire la population de moitié et d'éliminer au moins trois mille femelles. Solution d'autant plus facile que l'organisation de la mise en conserve, à Port-Menier, permettait de tout récupérer. La recommandation a paru trop énergique. Une neige abondante, l'hiver suivant, a détruit plusieurs milliers d'individus. La principale leçon à tirer de ces divers exemples, c'est qu'une protection du gibier bien comprise exige néanmoins une chasse normale pour éliminer le surplus qui mettrait en péril l'équilibre naturel.

Entités nouvelles et corrections

1. *Allium Schoenoprasum* L. var. *laurentianum* Fern.
f. *albiflorum* Rousseau, n.f.

A var. laurentiano recedit floribus albis.

ANTICOSTI: Rivière au Saumon, environ 15 milles de la mer, corniches calcaires. 11 juillet 1942. *Rousseau 52211.* TYPE dans l'Herbier Marie-Victorin, Jardin botanique de Montréal et Université de Montréal.—Rivière

Vauréal, sur les talus calcaires en amont de la chute. 8 juillet 1942. *Rousseau 52115.*—Rivière Vauréal, près de la source, environ 14 milles en amont de la chute, sur berge graveleuse. 10 juillet 1942. *Rousseau 52155.*—Crique à la Chaloupe, à 7 milles de la mer, sur platières graveleuses. 14 juillet 1942. *Rousseau 52279.*—Crique à la Chaloupe, à environ 15 milles de la mer, berge graveleuse calcaire. 13 juillet 1942. *Rousseau 52270.*—Rivière McKane, platières à l'embouchure. 16 juillet 1942. *Rousseau 52304.*

Le polymorphisme de l'*Allium Schoenoprasum* est bien connu. Vvedensky (63), Levan (32), Turesson (61) et Hylander (30), notamment, en ont fait état. Le matériel indigène de la province de Québec est généralement partagé en deux variétés: *Allium Schoenoprasum* L. var. *sibiricum* (L.) Hartm. et *A. Schoenoprasum* L. var. *laurentianum* Fernald (16). Cette dernière se distingue de la précédente par ses fleurs plus petites (8–10 mm. long au lieu de 10–14 mm.), par ses fleurons plus colorés et moins atténués, par le pédicelle plus court et l'ombelle moins développée (2.3–3 cm., rarement 3.3 cm.). Quand on en vient à l'application des caractères, toutefois, le problème n'est pas toujours simple. Il n'y a aucun doute que l'*Allium Schoenoprasum* doit être entièrement étudié de nouveau. La description du var. *laurentianum* ne fait aucun état du port de la plante. Sur les grèves estuariennes d'eau douce du Saint-Laurent, où abonde l'*Allium Schoenoprasum*, de même qu'au Bic, où il en a une colonie, les plantes croissent en touffes comme chez la variété cultivée dans les jardins. Les spécimens d'Anticosti poussent isolément ou presque et comme ils ont de 50 à 60 cm. de longueur, ils ressemblent plus à des oignons de jardin qu'à la ciboulette (fig. 10). Les spécimens d'herbier, sans doute, nous renseignent mal sur le port de la plante, mais les entités ne sont pas décrites seulement pour être reconnues en herbier. Le fait d'être reliés en touffes ou de pousser isolément est un caractère fondamental chez les *Allium* non seulement pour distinguer des variétés, mais même des espèces. Un autre caractère à envisager lors de la revision de l'espèce, chez les spécimens de l'estuaire d'eau douce du Saint-Laurent, comme ceux d'Anticosti, c'est la tendance à produire des inflorescences hémisphériques au lieu de sphériques. Caractère de maniement difficile cependant, car les inflorescences sphériques débutent toujours par l'état hémisphérique. De même, les spécimens américains, ceux du Saint-Laurent surtout, sont plutôt pauciflores, comparés aux formes européennes.

Les spécimens d'Anticosti, même si la description des variétés n'est pas tout à fait satisfaisante, se rattachent mieux au var. *laurentianum* qu'au var. *sibiricum*. Toutefois, comme Fernald, dans sa description du var. *laurentianum*, insiste sur un caractère de coloration: "*a var. sibirico recedit floribus minoribus intense coloratis*", il y a lieu de distinguer par une entité nouvelle l'albino si fréquent sur l'Ile.

2. *Castilleja septentrionalis* Lindley var. *micmacorum* Rousseau, n. var.

Habitus et pubescentia Castillejae septentrionalis. Differt bracteis (20 mm. longis, 7 mm. latis; in var. typica: 25 mm. longis, 12 mm. latis) anguste ovato-acutis, integris aut leviter dentatis alboviridescentibus (lobatis et purpureis in var. typica).

Pour la pubescence et les caractères généraux, comme le *Castilleja septentrionalis* typique. Il en diffère surtout par les bractées (20 mm. long., 7 mm. larg. en moyenne) d'habitude étroitement ovales-aiguës, entières ou légèrement dentées, blanc verdâtre, tandis que celles du *C. septentrionalis* typique sont larges (25 mm. long. par 12 mm. larg. en moyenne), nettement lobées et purpurines. PROVINCE DE QUÉBEC (ET FRONTIÈRE QUÉBEC–NOUVEAU-BRUNSWICK): Vallée de la Restigouche et de la Matapédia. Milnikek, river bank, Aug. 12–14, 1929. *Rousseau 32429.* TYPE dans l'herbier Marie-Victorin, Université de Montréal et Jardin botanique de Montréal.—Parmi les spécimens de cette variété citons: Junction of Restigouche and Patapedia river, gravelly flats, July 23–24, 1929. *Rousseau & Bonin 32156.*—Restigouche river, 5 to 10 miles below Patapedia river, July 24–25, 1929. *Rousseau & Bonin 32202.*

Sous le nom de *Castilleja septentrionalis*, Lindley publia en 1825 (33) une espèce nouvelle, observée en culture en Angleterre, dans un tapis de végétation herbacée apporté du Labrador. Le dessin colorié qui accompagne la description correspond assez bien aux plantes d'Anticosti, mais pas à celles de la Restigouche et de la Matapédia. La plante, sur le dessin de Lindley, est paniculée, ce qui est très rare chez cette espèce et représente un cas extrême. D'autre part, les spécimens d'Anticosti sont un peu plus fortement teintés de pourpre que sur le dessin. Il n'y a pas lieu néanmoins de créer un cadre taxonomique nouveau pour distinguer le type biologique de l'espèce du type taxonomique. La plante de la Matapédia et de la Restigouche, par contre, diffère suffisamment pour en faire une entité nouvelle. N'étaient les spécimens intermédiaires des Shikshoks et des rivières de la Gaspésie, on pourrait faire de la variété de la Restigouche une espèce nouvelle. Comme la région de la Restigouche constituait une partie du pays des Micmacs, dont il reste encore une réserve à Restigouche, il a semblé intéressant de leur dédier la variété.

Parmi les spécimens les plus représentatifs du *Castilleja septentrionalis* Lindley typique, citons les suivants de l'île d'Anticosti: rivière Jupiter, prairie marine au bord de la mer, 2 juillet 1942, *Rousseau 52003.*—Rivière Vauréal, sur les talus calcaires en amont de la chute, 8 juillet 1942. *Rousseau 52113.*—Rivière Pavillon, sur les platières et la berge graveleuse à l'embouchure, 17 juillet 1942. *Rousseau 52316.* Parmi les caractères du *C. septentrionalis* typique dont il faut se méfier, il y a la couleur pourpre des bractées. Celle-ci disparaissant souvent sur les spécimens d'herbier, seules les notes prises par le collecteur peuvent être de quelque service.

Gray, en 1876 (23), avait rattaché le *C. septentrionalis* au rang variétal sous *C. pallida* L. et caractérisait ainsi sa variété: "*var. septentrionalis commonly less pubescent, often almost glabrous, a span to two feet high: bracts not rarely tinged with purple; corolla two-thirds to three-fourths of an inch long; its lower lip less large, from one-third to half the length of the upper.*" Le *C. septentrionalis* Lindley et le *C. pallida* Lindley semblent suffisamment différents pour être distingués spécifiquement. D'autre part, il est possible aussi que le var. *septentrionalis*, tel que compris par Gray, soit presque l'analogue de la nouvelle

var. *micmacorum*. Même s'il en était ainsi, un nouveau nom s'imposerait pour que l'épithète variétale ne répète pas simplement l'épithète spécifique.

Pennell (50) a décrit pour l'ouest de l'Amérique plusieurs sous-espèces de *C. pallida*, mais aucun ne correspond à la plante de la Restigouche.

3. ×*Iris Sancti-Cyri* Rousseau, n. hybr.

Petalis triplo brevioribus sepalis, oblanceolatis, mediis Iridis Hookeri Iridisque versicoloris.

Pétales ayant approximativement le tiers de la longueur des sépales, oblancéolés, intermédiaires entre les pétales extrêmement réduits de l'*Iris Hookeri* Penny (connu un temps sous le nom d'*Iris setosa* Pall var. *canadensis* Foster) et ceux, très développés de l'*Iris versicolor* L. Les deux espèces, qui sont incontestablement les parents de l'*Iris Sancti-Cyri*, poussaient d'ailleurs dans la localité-type du nouvel hybride (fig. 11). Cette entité est dédiée à Napoléon-Dominique Saint-Cyr (1826–1899), conservateur du musée du département de l'Instruction publique, au siècle dernier, qui herborisa sur Anticosti et la Côte-Nord et publia quelques études sur ses herborisations. ANTICOSTI: rivière à la Loutre, dans la tourbière, près de l'embouchure. 3 juillet 1942. *Rousseau 52043*. TYPE dans l'herbier Marie-Victorin, Jardin botanique de Montréal et Université de Montréal.

4. *Iris versicolor* L. f. *albocaerulea* Rousseau, n.f.

A typo differt petalis stigmatibusque albis, leviter caeruleotinctis.

Les fleurs de cette forme sont bicolores. Les sépales pétaloïdes (les pièces les plus développées de la corolle) sont bleus, comme chez les spécimens normaux, tandis que les stigmates pétaloïdes (en face des sépales) et les pétales, alternant avec les sépales, sont blancs, avec une légère teinte bleutée. Il s'agit donc d'un albino partiel. Le véritable albino est *I. versicolor* L. f. *Murrayana* Fern. (19). ANTICOSTI: rivière au Saumon, entre la source au centre de l'Ile et le gouffre McCormick, à 6 milles de la source (37 milles de la mer), sur rivage calcaire. 10 juillet 1942. *Rousseau 52185*. TYPE dans l'Herbier Marie-Victorin, Jardin botanique de Montréal et Université de Montréal.

5. *Listera cordata* (L.) R. Br. f. *disjuncta* Lepage, emend. Rousseau

Dans la description de cette forme, l'abbé Ernest Lepage (31) attribue à la feuille inférieure un contour normal et à la supérieure, plus petite, la forme ovée. Le spécimen 52065, rapporté de la rivière à l'Huile, a également une feuille supérieure plus petite, mais de contour normal. Il n'y a pas lieu de distinguer cette forme de celle de l'abbé Lepage, d'autant plus qu'il est impossible de savoir, dans un cas comme dans l'autre, s'il s'agit d'une mutation ou d'une simple anomalie de croissance. Quoiqu'il en soit, il y a lieu de distinguer le type à feuilles alternes. Il faut donc amender légèrement la description du f. *disjuncta* Lepage pour lui donner un sens un peu plus étendu. La description devrait se lire: "*A typo differt foliis alternis.*"

6. *Parnassia caroliniana* Michx f. *reducta* Rousseau, n.f.

A normali Parnassia caroliniana recedit duobus staminodibus praeter quidque petalum.

ANTICOSTI: rivière Jupiter, platière herbeuse à l'embouchure de la rivière, 21 juillet 1942. *Rousseau 52406.* TYPE dans l'Herbier Marie-Victorin, Jardin botanique de Montréal et Université de Montréal.—Rivière au Saumon, dans la tourbière près de l'embouchure, 2 août 1925. *Marie-Victorin, Rolland, Louis-Marie 21781.*

Chez les *Parnassia*, devant chaque pétale, se trouve un groupe de staminodes ou étamines rudimentaires, reliés à la base. Bien que Michaux n'en fasse aucunement mention dans sa description, le *Parnassia caroliniana* compte habituellement de trois à cinq staminodes devant chaque pétale. Dans la présente forme, le nombre habituel de staminodes est de deux devant chaque petale.

7. *Senecio gaspensis* Greenm. var. *Victorini* Rousseau, n. var.

Planta 3–6 dm. alta, caule tomentoso in axilla foliorum, foliorum basilarium laminis (2–12 cm. longis, 1–3.5 cm. latis, petiolis 6–14 cm. longis) infra cinereopubescentibus, irregulariter serratis, subincisis; foliorum caulinorum laminis basi pinnatifidis, apice generaliter dentatis; capitulis siccis 7–10 mm. latis, bracteis 5–6 mm. longis, obovatis, apice ciliatis, floribus tubulatis 6–7.5 mm. longis, tubula longiore quam parte campanulata, lobis divergentibus; achaeniis 2.5–2.8 mm. longis, glabris, apice ornatis spiculo exserto.

ANTICOSTI: rivière Chicotte, talus calcaires, dans les gorges, 15 août 1926. *Victorin & Rolland 25163.* (Distribué sous le nom de *Senecio plattensis* Nutt.) TYPE dans l'herbier Marie-Victorin, Jardin botanique de Montréal et Université de Montréal.—Rivière Chicotte, sur la berge à l'orée du bois, 17 juillet 1942. *Rousseau 52356.*—Rivière MacDonald, sur les platières calcaires. 24 juillet 1925. *Victorin, Rolland, Louis-Marie 21285.* (Distribué sous le nom de *Senecio pauperculus* var. *Balsamitae*). Variété dédiée au F. Marie-Victorin qui, le premier, a attiré l'attention sur les seneçons de la rivière Chicotte. *Victorin* se disant en latin *Victorinus* et non *Victorinius*, l'épithète génitive *Victorini* convient mieux que *Victorinii*, recommandée à tort par les règles internationales de la nomenclature et rendant plus hommage à l'esprit de système qu'au bon goût.

Au cours d'expéditions sur l'île d'Anticosti, le F. Marie-Victorin et le F. Rolland-Germain trouvèrent dans les gorges de la rivière Chicotte un seneçon aberrant qu'ils identifièrent d'abord au *Senecio plattensis* Nutt., poussant du sud de l'Ontario au Texas. Plusieurs caractères néanmoins distinguent la plante d'Anticosti du *Senecio plattensis* vrai. Ce dernier a des tiges lanigères et des feuilles pubescentes-cendrées; des bractées involucrales (5 mm. long) légèrement obovées et à sommet acuminé, et parfois un peu ciliées; des fleurs tubuleuses (4-4.5 mm. long) à partie tubuleuse plus courte (environ 2 mm. long.) que la partie campanulée (env. 2.5 mm. de long. dans une fleur de 4.5), à corolle non évasée dans la partie libre; des achaînes à arêtes ciliées (à papilles claviformes), terminées au sommet par une petite pointe cachée dans la dépression cratériforme. La plante d'Anticosti (var. *Victorini*), par contre, a des tiges très légèrement lanigères, des feuilles moins pubescentes-cendrées;

des bractées (5–6 mm. long) obovées, ciliées au sommet; des fleurs tubuleuses (6–7.5 mm. long.) à tube plus long (3.5 mm. dans une fleur de 6.5 mm. long.) que la partie campanulée de la corolle, et à lobes évasés; des achaînes (2.5–2.8 mm. long) glabres, terminées par une pointe aiguë dépassant nettement le rebord de la dépression cratériforme terminale.

D'autre part, le *Senecio gaspensis* Greenm. typique (25) a des tiges floculeuses-tomenteuses dans l'axe des feuilles, des feuilles (0.5–8 cm. long. par 1–3.5 cm. larg., pétioles 1–12 cm.•long.) glabres sur les deux faces (sauf chez les jeunes plantes) et crénelées-dentées. Le *Senecio gaspensis* var. *Victorini* diffère donc du *Senecio gaspensis* typique surtout par les feuilles légèrement pubescentes-cendrées, à dents serrées, assez profondément incisées.

8. ×*Solidago Raymondi* Rousseau, n. hybr.

Planta 12–40 cm. longa, caule puberulenti, tamen laxe pubescenti; reliquis fibrosis foliorum anteriorum instructa; foliis imis 1.5–5 cm. longis, oblanceolatis leviter dentatis; foliis mediis 3–8 cm. longis, anguste oblanceolatis, longe attenuatis in petiolo alato, integris vel subintegris; foliis inflorescentiae linearibus vel lanceolatis 1–2 cm. longis; inflorescentia 2–12 cm. longa plus minusve ramificata; bracteis involucri 2–3 vel 3–4-seriatis, omnibus glutinoso-pruinosis, generaliter linearibus acutis vel subacutis, tamen leviter spathulatis subacutis; pilis achaeniarum appressis, vel obliquis, vel erectis.

Plante 12–40 cm. long., à tige pubérulente, portant aussi parfois en outre une pubescence lâche; base de la tige conservant les restes fibreux des anciennes feuilles basilaires; feuilles de la base 1.5–5 cm. long., oblancéolées, légèrement dentées-serrées; feuilles du centre de la tige 3–8 cm. long., étroitement oblancéolées, atténuées en un long pétiole ailé, *entières ou presque*; feuilles de l'inflorescence linéaires ou lancéolées, 1–2 cm. long., entières; inflorescence 2–12 cm. long. plus ou moins ramifiée; bractées de l'involucre en 2–3 ou 3–4 séries, *glutineuses-pruineuses* sur toute la surface externe, généralement linéaires et à pointe aiguë ou subaiguë, parfois légèrement spathulées et à pointe subaiguë; poils des achaînes appliqués, ou obliques ou hirsutes. ANTICOSTI: rivière Pavillon, sur les platières et la berge graveleuse à l'embouchure, 17 juillet 1942. *Rousseau 52325.* TYPE dans l'herbier Marie-Victorin, Jardin botanique de Montréal et Université de Montréal.—Rivière Chicotte, talus en amont des gorges, 17 juillet 1942. *Rousseau 52355.*—Rivière Chicotte, corniches calcaires des gorges près de l'embouchure, 17 juillet 1942. *Rousseau 52345A.*—Rivière Jupiter, platière herbeuse à l'embouchure de la rivière, 21 juillet 1942. *Rousseau 52407.* Hybride dédié à Marcel Raymond, qui a étudié tout particulièrement les matériaux floristiques rapportés d'Anticosti depuis de nombreuses années et qui a apporté au présent travail une collaboration des plus actives.

Le problème du *Solidago humilis* Pursh et des espèces affines a été, à quelques reprises, l'objet de discussions, notamment par Fernald (15, 21, 21*a*) et Cronquist (6). Cette discussion met en cause plusieurs espèces apparentées, *Solidago uliginosa* Nutt., *S. racemosa* Greene, *S. Purshii* Porter (*S. chrysolepis* Fernald), S. *Randii* (Porter) Britton et autres.

Plusieurs botanistes, dont Fernald avaient l'habitude d'assimiler au *S. racemosa* Greene (24) des spécimens des corniches calcaires et des platières de

l'embouchure des rivières d'Anticosti. Ces plantes correspondent pourtant peu à la description originale de Greene et si elles se rapprochent du *Solidago-Purshii* Porter (*Solidago chrysolepis* Fernald) (14 et 21*a*) elles ne répondent pas non plus pleinement à sa description.

D'autre part, Fernald, en 1927, décrivait une espèce nouvelle, *Solidago Victorinii* (17) caractérisée notamment par des bractées pruineuses, à sommet légèrement arrondi et par des achaînes à poils hirsutes. Il n'y a pas lieu ici de discuter du statut des deux entités et surtout du problème taxonomique *S. racemosa* vs. *S. Purshii*, mais de signaler plutôt la présence de formes de transition entre *S. Victorinii* et le soi-disant *S. racemosa*. Pour éviter toute confusion, ce que l'on considère à tort ou à raison comme *S. racemosa* Greene dans la flore d'Anticosti est parfaitement représenté par le spécimen suivant: Rivière Jupiter, talus argilo-calcaire sec, au 6ième mille, 22 juillet 1942, *Rousseau 52427*. Non seulement les spécimens décrits plus haut comme ×*Solidago Raymondi* sont intermédiaires entre le *Solidago Victorinii* et le *Solidago racemosa* de la Jupiter (No 52427), mais ils présentent mêmes tous les symptômes de la ségrégation mendélienne. En effet, si tous les quatre ont des feuilles presque entières et des bractées glutineuses et pruineuses, ils répondent d'autre part aux descriptions suivantes. No 52325: bractées en 3 séries aiguës; poils appliqués sur les achaînes. No 52355: bractées en 2–3 séries linéaires, non spatulées, obtuses; poils de l'achaîne ni appliqués, ni hirsutes, mais intermédiaires. No 52345A: bractées en 2–3 séries, légèrement spatulées, étroites, à pointe subaiguë; achaînes à poils hirsutes. No 52407: bractées en 3–4 séries, aiguës; achaînes presque glabres, à poils appliqués. Cet hybride présumé est assez fréquent pour mériter un rang taxonomique. Le ×*Solidago Raymondi* possède comme caractère constant du *S. Victorinii* l'état pruineux-glutineux des bractées et comme caractères accidentels de cette espèce la forme oblonque-spatulée des bractées et les achaînes à poils hirsutes de certains spécimens. D'autre part, le ×*S. Raymondi* retient du *S. racemosa* (du moins tel que représenté par le spécimen 52427) le port, les feuilles entières ou presque, les bractées généralement linéaires aiguës ou subaiguës, parfois les achaînes à poils appliqués. Enfin les deux plantes réputées parentes, poussant côte à côte sur les platières et les talus de deux rivières d'Anticosti au moins, on s'explique qu'il puisse se produire facilement des hybrides, d'autant plus que les *Solidago* sont visités par des insectes, notamment des bourdons.

9. *Tanacetum huronense* Nutt. var. *bifarium* Fern f. *lanatum* Rousseau, n.f.

A var. bifario recedit foliis caulibusque lanatis.

ANTICOSTI: rivière McKane, platières à l'embouchure, 16 juillet 1942. *Rousseau 52303*. TYPE dans l'herbier Marie-Victorin, Jardin botanique de Montréal et Université de Montréal.

Presque tout le matériel d'Anticosti de *Tanacetum huronense* se place dans le var. *bifarium* Fernald (18). C'est d'ailleurs de cette île que vient le spécimen-type de la variété. Au cours du voyage de 1942 toutefois, la rivière

McKane a fourni des spécimens tellement laineux qu'il y a lieu de les distinguer au rang de forme. Une récolte de la rivière Pavillon (*Rousseau 52323*), d'autre part, se rattache nettement au var. *terrae-novae* Fernald et d'autres récoltes présentent des formes de transition entre var. *bifarium* et var. *terrae-novae*, mais il n'y a pas lieu de les distinguer taxonomiquement.

10. *Triglochin palustris* L. f. *Ferlandiana* Rousseau, n.f.

A var. typica recedit fructibus apice non strangulato.

PROVINCE DE QUÉBEC: Rivière Jupiter (Anticosti), talus argilo-calcaire au 6e mille. 22 juillet 1942. *Rousseau 42422.* TYPE dans l'herbier Marie-Victorin, Jardin botanique de Montréal et Université de Montréal.—Saint-Adélard, co. Gaspé, sur un fond marneux d'un étang desséché, 6 août 1931. *Victorin, Rolland, Jacques 44871.*

Le matériel européen typique de *Triglochin palustris* a des fruits munis d'un étranglement près de l'extrémité. Dans le matériel de l'est du Canada, la constriction est à peine visible ou fait entièrement défaut. La nouvelle forme décrite présentement correspond à un pôle de l'espèce. Variété de peu d'importance sans doute, mais qui a au moins la valeur du *Menyanthes trifoliata* var. *minor* et d'autres formes américaines différant quelque peu du type européen. Cette forme est dédiée à l'abbé J.-B.-A. Ferland, naturaliste amateur et historien, qui visita Anticosti au milieu du siècle dernier (5, 12, 13, 22). Il faudra éviter de confondre l'épithète *Ferlandiana* avec *Fernaldiana*, qui, pour cause, est plus fréquente dans la littérature botanique.

11. *Viola affinis* LeConte var. *subarctica* Rousseau, n. var.

A Viola affine typica recedit rhizomatibus tenuibus.

PROVINCE DE QUÉBEC: Rivière à la Loutre (Anticosti), platières à l'embouchure de la rivière, 2 juillet 1942. *Rousseau 52023.* TYPE dans l'herbier Marie-Victorin, Jardin botanique de Montréal et Université de Montréal.—Rivière Vauréal (Anticosti), à 1–2 milles en amont de la chute, sur les platières herbeuses, 9 juillet 1942. *Rousseau 52122.* Anse aux Fraises, près Port-Menier (Anticosti), sur la berge humide, 4 juillet 1942. *Rousseau 52007.*—Causapscal (rivière Matapédia), gravelly beach, Aug. 12–14, 1929. *Rousseau 32392.*—Montagne Blanche (Rimouski), tourbière, 23 juin 1938. *Lepage 331.*—ONTARIO: Moosonee, talus herbeux au bord de la rivière Moose, 51° 15′ N., 26 juin 1944. *Dutilly et Lepage 12035.*

Dans l'état actuel de nos connaissances, l'aire générale de *Viola affinis* s'arrête à la région de Montréal et d'Ottawa. Les localités excentriques comprennent: la vallée de la Matapédia, la montagne Blanche, Anticosti et le fond de la baie James. Or il se trouve que tous ces spécimens boréaux qui, par leurs feuilles longuement aiguës et presque atténués, sont du *Viola affinis*, en diffèrent beaucoup toutefois par leurs rhizomes très élancés, quand le *V. affinis* typique a des rhizomes charnus. On sait l'importance de ce caractère

chez les violettes acaules: il permet même de séparer des groupes d'espèces bien différentes. Si l'on s'en remet à nos connaissances actuelles sur la distribution du *Viola affinis* il y aurait donc discontinuité nette entre l'aire du type et celle du var. *subarctica*.

Liste des plantes récoltées ou observées au cours des voyages de 1940 et 1942

A part de courtes notes, l'auteur ne devait publier aucun relevé floristique de la flore d'Anticosti. Ses matériaux rapportés du centre de l'Ile en 1940 et 1942 devaient être incorporés au travail d'ensemble du F. Marie-Victorin. C'est ainsi qu'ils auraient eu le maximum de rendement. La mort accidentelle du F. Marie-Victorin ayant mis un terme au projet initial, il a semblé utile de rassembler en une liste les plantes ayant fait l'objet d'observations au cours des deux voyages. D'autant plus que la dernière liste un peu élaborée de la flore d'Anticosti a déjà un demi-siècle (60).

Il faut signaler cependant que les récoltes de 1940 et 1942 avaient été délibérément fragmentaires. Le F. Marie-Victorin et le F. Rolland-Germain, ayant consacré plusieurs étés à l'exploration de l'Ile, il n'était pas question d'en entreprendre un relevé complet, mais de combler les vides. Beaucoup d'espèces communes manquent donc dans la présente liste. Ainsi, *Clintonia borealis*, *Cornus canadensis*, *Maianthemum canadense*, *Oxalis montana*, *Dryopteris spinulosa* etc. D'autres, uniformément répandues partout, ne sont mentionnées que pour une localité, car seuls les spécimens recueillis et les observations consignées au journal doivent entrer en ligne de compter. L'étude sur les *Carex d'Anticosti* que Marcel Raymond vient de terminer, et qui paraîtra sans doute incessamment, constitue la première tranche d'une étude systématique définitive de la flore de l'Ile. Grâce à ses soins, de précieux matériaux accumulés pendant des années pourront contribuer à l'enrichissement de nos connaissances.

Dans la liste qui suit, l'indication "platières" se rapporte aux platières graveleuses inondées au printemps, si fréquentes à l'embouchure des rivières d'Anticosti. Les talus ou berges indiqués comme argilo-calcaires sont peut-être parfois des formations marno-calcaires. Les "corniches calcaires" sont des falaises dont les strates horizontales émergent parfois comme des tablettes. Les tables calcaires sont également des formations horizontales, mais plutôt basses et à peine au-dessus du niveau de l'eau. Les prairies littorales sont des formations de grève au-dessus du niveau des hautes marées. Sauf indication contraire, les récoltes proviennent de l'embouchure des rivières.

Lycopodinées

Selaginella selaginoides (L.) Link. Berges graveleuses, corniches calcaires. Lac Wickenden, ruisseau Cailloux, rivière au Saumon (15 milles de la mer), Chicotte.

Equisetinées

Equisetum arvense L. var. *boreale* (Bong) Rupr. f. *pseudo-alpestre* Vict. Berge graveleuse. Lac Wickenden.

Equisetum fluviatile L. Tourbière. Hauteur des terres entre source des rivières Vauréal, au Saumon, à la Chaloupe.

Equisetum littorale Kuehl. f. *arvensiforme* (AA. Eaton) Vict. Berge graveleuse. Rivière Chicotte.

Equisetum palustre L. var. *americanum* Vict. Platières. Rivière à la Loutre.

Equisetum scirpoides Michx. Berge humide. Lac Wickenden, anse aux Fraises (près Port-Menier).

Equisetum sylvaticum L. Orée du bois. Crique à la Chaloupe.

Equisetum variegatum Schleicher. Platières graveleuses. Rivière Galiote, lac Wickenden.

Filicinées

Botrychium Lunaria L. Berge gazonnante. Port-Menier.

Botrychium Lunaria L. var. *subincisum* (Roeper). Milde. Prairie tourbeuse littorale. Rivière à la Loutre.

Pteridium aquilinum (L.) Kuhn var. *lanuginosum* (Bong) Fern (18). Bois clair, de type subalpin, à la hauteur des terres à la source des rivières au Saumon et à la Chaloupe. C'est la forme commune de l'ouest de l'Amérique, de l'Alaska à la Californie. Dans le Québec, on l'avait rencontrée dans la péninsule de Gaspé et dans le comté de Mégantic. Les autres plantes du Québec se placent généralement dans le *Pteridium latiusculum*. Les deux espèces sont en réalité très rapprochées et pour ma part, je ne suis pas sûr qu'il faille les maintenir distinctes. Le *P. latiusculum* déjà noté pour le lac Wickenden était sans doute plutôt de la présente variété.

Woodsia Belli (Lawson) A. E. Porsild, (Syn. *W. alpina* (Bolton) S. F. Gray pro parte). Corniches calcaires. Rivière Chicotte. Nouveau pour Anticosti.

Cystopteris bulbifera (L.) Bernh. Talus calcaire, berge graveleuse. Lac Wickenden.

Asplenium viride Huds. Talus calcaires. Rivière au Saumon.

Conifères

Pinus Strobus L. Platières au voisinage de la berge. Rivière Vauréal.

Larix laricina (Du Roi) Koch. Orée du bois, platières. Lac Wickenden, rivière Vauréal (embouchure).

Larix laricina (Du Roi) Koch. f. *depressa* Rousseau (52). Platières, tourbières. Rivières Jupiter (embouchure), Vauréal (23 milles de la mer), hauteur des terres entre les sources des rivières Vauréal, au Saumon et à la Chaloupe.

Picea glauca (Moench) Voss. Orée du bois. Lac Wickenden, rivière Jupiter (embouchure).

Picea glauca (Moench) Voss f. *parva* (Vict.) Fern. & Weath. Berge argilo-calcaire. Ruisseau Bilodeau.

Picea mariana (Mill.) BSP. Partout dans l'Ile.

Picea mariana (Mill.) BSP. f. *squamea* (Provancher) Marie-Victorin. Connu sous le nom de "p'tits Saint-Michel". Tourbières, bois clairs, berges. Rivière Vauréal (23 milles de la mer), hauteur des terres à la source des rivières Vauréal, au Saumon, à la Chaloupe.

Abies balsamea (L.) Mill. Orée du bois. Lac Wickenden.

Abies balsamea (L.) Mill. f. *hudsonia* (Bosc.) Fern. & Weath. Berge graveleuse. Rivière Vauréal (23 milles de la mer).

Juniperus communis L. var. *depressa* Pursh. Berge graveleuse, platières. Rivières Sainte-Marie, Vauréal (embouchure et 10 milles de la mer).

Juniperus horizontalis Moench. Platières, talus argilo-calcaires. Rivières Jupiter (embouchure), Sainte-Marie, Chicotte, Vauréal (embouchure), au Saumon (10 milles de la mer).

Thuja occidentalis L. Bosquet dans le bois de *Picea mariana*. Rivière Jupiter

Scheuchzériacées

Triglochin maritima L. Tourbières. Vauréal (9 milles de la mer).

Triglochin palustris L. Berge graveleuse. Lac Wickenden.

Triglochin palustris L. f. *Ferlandiana* Rousseau.* Talus argilo-calcaire, rivière Jupiter (6 milles de la mer).

Scheuchzeria palustris L. var. *americana* Fern. Tourbières. Hauteur des terres entre sources des rivières Vauréal, au Saumon, à la Chaloupe.

Naiadacées

Potamogeton epihydrus Raf. Lac Wickenden.

Potamogeton filiformis Pers. var. *borealis* (Raf.) St-John. Lac Plantain, lac Wickenden, rivières au Saumon (37 milles de la mer), Jupiter, à la Chaloupe (21 milles de la mer).

Potamogeton gramineus L. Lac Wickenden, rivières au Saumon (35 et 43 milles de la mer), Sainte-Marie, à la Chaloupe (21 milles de la mer).

Potamogeton panormitanus Biv.-Bern. Rivière au Saumon (35 milles de la mer).

Liliacées

Allium Schoenoprasum L. var. *laurentianum* Fernald. Berges graveleuses. Rivières Sainte-Marie, McKane, Jupiter, à la Chaloupe, Vauréal (depuis l'embouchure jusqu'à la source), au Saumon (toute la longueur), aux Plats, lac Wickenden. Probablement partout au bord des cours d'eau. Le rattachement des plantes anticostiennes au var. *laurentianum* Fernald, sans être tout

* *Pour description, voir supra.*

à fait satisfaisant, est du moins la solution qui convient le mieux. Plante atteignant de 50 à 60 cm. de haut, vivant par brins isolés et non en grosses touffes.

Allium Schoenoprasum L. var. *laurentianum* Fern. f. *albiflorum* Rousseau.* Partout où pousse le précédent.

Tofieldia glutinosa (Michx.) Pers. Berge argilo-calcaire, platières. Rivières à l'Huile, Jupiter, aux Plats, Vauréal (7 et 23 milles de la mer), hauteur des terres à la source des rivières Vauréal, au Saumon et à la Chaloupe.

Tofieldia pusilla (Michx.) Pers. Corniches calcaires, platières, rivières Jupiter, Chicotte.

Zigadenus glaucus (Nutt.) Nutt. Berges graveleuses, platières. Lac Wickenden, rivières Jupiter, aux Plats, Vauréal (7 milles de la mer), au Saumon (43 milles de la mer), à la Chaloupe (7 milles de la mer).

Iridacées

Iris Hookeri Penny. (Syn. *Iris setosa* Pall. var. *canadensis* Foster). Ligne littorale. Rivière à la Loutre, ruisseau Bilodeau.

×*Iris Sancti-Cyri* Rousseau.* (Hybride *Iris Hookeri* × *versicolor*). Tourbière. Rivière à la Loutre.

Iris versicolor L. Tourbières, rivages calcaires. Rivières à la Loutre, au Saumon (à 25 et 38 milles de la mer), Vauréal (9 milles de la mer).

Iris versicolor L. f. *albocaerulea* Rousseau.* Rivages calcaires. Rivière au Saumon (à 40 milles de la mer).

Sisyrinchium montanum Greene. Platières calcaires. Rivières à l'Huile, Vauréal (21 milles de la mer), au Saumon (37 milles de la mer), Jupiter, Pavillon.

Sisyrinchium montanum Greene var. *crebrum* Fernald. Platières graveleuses, tourbières, corniches calcaires. Rivières à la Loutre, Jupiter, Chicotte, à la Chaloupe (7 milles de la mer).

Joncacées

Juncus alpinus Vill. Typique. Berge graveleuse, talus argilo-calcaires. Rivières Vauréal (7 milles de la mer), Chicotte, Cailloux.

Juncus alpinus Vill. var. *alpestris* (C. J. Hartman) C. Hartman. Berge argilo-calcaire. Rivière Sainte-Marie. Inconnu dans le Québec ailleurs qu'en Minganie et sur Anticosti. Dans le secteur arctique du Québec, existe sous une forme réduite à un seul capitule, f. *uniceps* Krok & Lagerstedt (fide Marcel Raymond). Voir Lindquist (34).

Juncus alpinus Vill. var. *fuscescens* Fern. Rivages calcaires. Rivière au Saumon (43 milles de la mer).

Juncus alpinus Vill. *rariflorus* (E. Fries) C. J. Hartman. Grève. Lac Wickenden, rivière Jupiter (6 milles de la mer).

* *Pour description, voir supra.*

Juncus balticus Willd. var. *littoralis* Engelm. Talus argilo-calcaires. Rivière Jupiter (6 milles de la mer).

Juncus nodosus L. Grève calcaire. Lac Wickenden.

Cypéracées

Eleocharis acicularis (L.) R. & S. Platière. Embouchure de la Jupiter.

Eleocharis capitata (L.) R. Br. Berge graveleuse. Lac Wickenden.

Eleocharis pauciflora (Lightf.) Link. Berge graveleuse. Lac Wickenden.

Eleocharis pauciflora (Lightf.) Link. var. *Fernaldii* Svenson. Platières. Rivières Chicotte, à la Chaloupe (7 milles de la mer).

Scirpus cespitosus L. var. *callosus* Bigel. Talus calcaire, berge graveleuse. Rivière Vauréal (7 milles et 21 milles de la mer).

Scirpus hudsonianus (Michx.) Fern. Tourbières, grèves graveleuses. Lac Wickenden, hauteur des terres entre rivières Vauréal, au Saumon et crique de la Chaloupe.

Scirpus rubrotinctus Fern. Corniches calcaires. Rivière au Saumon (10 milles de la mer).

Eriophorum angustifolium Honckeny. 1782. (Syn. *E. angustifolium* Roth. 1788.) Tourbière. Hauteur des terres entre rivière au Saumon et rivière à la Chaloupe.

Eriophorum brachyantherum Trautv. & Meyer. Tourbières ou bords tourbeux des rivières. Rivière à l'Huile, centre de l'île entre le 10e mille de la rivière au Saumon et la source du crique à la Chaloupe. Nouveau pour Anticosti. Déjà connu de Terre-Neuve et de l'Abitibi (Québec) sous le nom d'*E. opacum* (Björn.) Fern.

Eriophorum Chamissonis C. A. Meyer. (Syn. *E. russeolum* Fries). Tourbière, berge vaseuse. Centre de l'Ile à la source des rivières Vauréal et au Saumon, rivière à l'Huile.

Eriophorum viridicarinatum (Engelm.) Fern. Tourbières, berges graveleuses. Rivières Vauréal (à 9 milles de la mer), au Saumon (à 25 milles de la mer), au centre de l'île (à la source des rivières Vauréal et au Saumon), rivière à la Chaloupe (8–9 milles de la mer).

Kobresia simpliciuscula (Wahl.) Mack. Platières. Rivière Jupiter.

Carex. M. Marcel Raymond les cite tous dans une étude qui paraîtra bientôt dans le Can. J. Research.

Graminées

Glyceria striata (Lam.) Hitchc. Platières, talus argilo-calcaires, grèves. Lac Wickenden, rivières Vauréal (7-9 milles de la mer), au Saumon (43 milles de la mer), à la Chute.

Poa alpina L. Platières, talus calcaires. Rivière Vauréal (7 milles de la mer), ruisseau Poulin, rivière Pavillon.

Poa costata Schum. Berge. Rivière Sainte-Marie.

Poa glauca Vahl. Berge. Rivière Sainte-Marie.

Poa laxa Haenke. Talus argilo-calcaires. Rivière au Saumon (à dix milles de la mer).

Poa saltuensis Fern. & Wieg. Prairie en bordure de la rivière. Rivière au Saumon (35 milles de la mer).

Festuca prolifera (Piper) Fern. var. *lasiolepis* Fern. Corniches calcaires. Rivières au Saumon (à 10 milles de la mer), Chicotte.

Festuca rubra L. Talus argilo-calcaires, prairies littorales. Port-Menier, rivières Sainte-Marie, à la chute, Vauréal.

Bromus ciliatus L. Orée du bois et grève, lac Wickenden.

Schizachne striata (Michx) Hulten (29) [syn. *S. purpurascens* (Torr.) Swallen.] Lac Wickenden, rivières à l'Huile, Vauréal (21 milles de la mer).

Danthonia sp. Nouvelle espèce étudiée par F. Rolland-Germain. Platières et talus calcaires. Rivières Vauréal (à 21 milles de la mer), au Saumon (à 10, 15 et 40 milles de la mer), ruisseau Poulin, crique à la Chaloupe (7 milles de la mer), rivière Jupiter (à l'embouchure).

Deschampsia caespitosa (L.) Beauv. Platières et corniches calcaires. Rivières au Saumon (10 milles de la mer), à la Chaloupe (7 milles de la mer).

Trisetum spicatum (L.) Richt. var. *molle* (Michx.) Beal. Talus calcaires. Rivière Vauréal (7 milles de la mer).

Trisetum spicatum (L.) Richt. var. *pilosiglume* Fern. Corniches calcaires, talus argilo-calcaires. Rivières au Saumon (10 milles de la mer), Chicotte.

Elymus mollis Trin. Grève halophytique. Port-Menier.

Hordeum jubatum L. Ballast. Port-Menier.

Agropyron repens (L.) Beauv. Grève. Lac Wickenden.

Agropyron repens (L.) Beauv. f. *aristatum* (Schum.) Holmb. Grève sablonneuse. Port-Menier. Cette plante étant celle qui poussait dans le voisinage de l'*Agroelymus Adamsii*, il est probable que ce soit elle et non l'*Agropyron repens* typique qui entre dans la composition de l'hybride bigénérique d'Anticosti.

Agropyron trachycaulum (Link.) Malte. Prairie herbeuse. Rivière au Saumon (35 milles de la mer).

× *Agroelymus Adamsii* Rousseau (55) (Hybride générique *Agropyron repens* × *Elymus mollis*). De Cugnac (7) a décrit l'hybride expérimental *Agropyron caninum* × *Elymus arenarius* sous le nom d'*Agroelymus Piettei*. Auparavant, on avait décrit en 1904, sous le nom de *Tritordeum Bergrothii* Lindb. fil. (*Medd. Soc. F. Fl. Fenn*, 32 : 21. 1904), cité par Holmberg (27), l'hybride *Agropyron repens* × *Elymus arenarius* de la Carélie pomerique. Il est possible que ce dernier hybride soit le même que celui de Cugnac. D'autre part, le nom *Tritordeum* a peut-être priorité sur *Agroelymus*, ce qu'il n'a pas été possible de vérifier en l'absence de la description originale du genre hybride *Tritordeum*.

Calamagrostis canadensis (Michx.) Beauv. Grève, Lac Wickenden.

Calamagrostis inexpansa Gray var. *brevior* (Vasey) Fern. Platières. Rivière McKane.

Calamagrostis neglecta (Ehrh.) Gaertn. Talus argilo-calcaires. Rivière à la chute.

Agrostis geminata Trin. f. *exaristata* Fern. Platières. Rivière Galiote.

Agrostis scabra Willd. Berge du lac Wickenden.

Phleum pratense L. Platières. Rivière à l'Huile.

Muhlenbergia glomerata (Willd.) Trin. var. *cinnoides* (Link) Hermann. Platières, grèves calcaires. Lac Wickenden. Rivière Jupiter.

Muhlenbergia Richardsonis (Trin.) Rydb. Platières. Rivières Vauréal (8–9 milles de la mer), Jupiter, Galiote.

Oryzopsis asperifolia Michx. Tourbière. Rivière Galiote.

Hierochloe odorata (L.) Beauv. var. *fragrans* (Willd.) Fern. Forêt claire. Rivière à l'Huile.

Graphephorum melicoideum (Michx) Beauv. Grève. Lac Wickenden.

Orchidées

Cypripedium Calceolus L. f. *rupestre* Vict. & Rousseau (47). Prairie littorale. Rivière Jupiter.

Orchis rotundifolia Banks. Bois clair, berge à l'orée du bois. Lac Wickenden, rivières à l'Huile, à la Loutre.

Habenaria dilatata (Pursh) Gray. Tourbières, berge graveleuse calcaire, talus argilo-calcaires, prairies littorales. Lac Wickenden, rivières Jupiter (embouchure), Chicotte, Vauréal (17 milles de la mer), hauteur des terres à la source des rivières Vauréal, au Saumou, à la Chaloupe.

Habenaria obtusata (Pursh) Richards. Bois, berge de rivière à l'orée du bois. Rivières Sainte-Marie, à la Loutre.

Corallorhiza trifida Chatelain. Bois. Rivières à la Loutre, à la Chute.

Arethusa bulbosa L. Tourbières et bois tourbeux. Hauteur des terres à la source de la Vauréal et de la rivière au Saumon, entre la rivière au Saumon et la rivière à la Chaloupe, et rivière à la Chaloupe (8 milles de la mer). Le type d'*Arethusa bulbosa* provient de la Virginie. La plante s'étend depuis la Caroline du Nord jusqu'à Terre-Neuve et Anticosti et pénètre jusqu'au Minnesota. Dans le Québec, outre Anticosti, où Marie-Victorin et Rolland-Germain l'ont déjà récoltée à deux reprises le long de la rivière la Loutre, elle n'existe qu'à Hatley, comté de Stanstead à Lanoraie, comté de Berthier, et à Oka, comté des Deux-Montagnes, dans des tourbières. (Fide Raymond.)

Spiranthes Romanzoffiana Cham. & Schlecht. Grève graveleuse. Lac Wickenden.

Malaxis unifolia Michx. Berge argilo-calcaire. Rivière Sainte-Marie.

Listera cordata (L.) R. Br. Bois de *Picea*, bosquet de *Thuja*. Rivières à la Loutre, Jupiter, à la Chute.

Listera cordata (L.) R. Br. f. *disjuncta* Lepage (31). Forêt claire, embouchure de la rivière à l'Huile.

Sparganiacées

Sparganium minimum Fries. Rivière Sainte-Marie.

Betulacées

Betula Michauxii Spack. (Syn. *Betula terrae-novae* Fernald). Pour les raisons d'abandonner le nom *B. terrae-novae*, voir Rousseau et Raymond (59). Tourbières du centre de l'Ile entre la source de la Vauréal et de la rivière au Saumon, et entre la rivière au Saumon et la rivière à la Chaloupe. Nouveau pour Anticosti. Déjà connu de Terre-Neuve et de la Pointe aux Esquimaux. En 1947, l'auteur l'a retrouvé au bord du lac Hubbard, à la source de la rivière George et sur les Monts Otish.

Betula pumila L. Berges graveleuses, tables calcaires, tourbières. Ruisseau Cailloux, rivière Galiote, rivière Vauréal (21 milles de la mer), lac Wickenden, hauteur des terres à la source des rivières Vauréal, au Saumon et à la Chaloupe.

Myricacées

Myrica Gale L. Berge argilo-calcaire, prairies tourbeuses. Lac Wickenden, rivières Vauréal (23 milles de la mer), au Saumon (25 et 43 milles de la mer), à la Chaloupe (embouchure), hauteur des terres à la source des rivières Vauréal, au Saumon, à la Chaloupe.

Salicacées

Populus tremuloides Michx. var. *rhomboidea* Vict. Berge calcaire. Rivière Vauréal (17 milles de la mer). Rare.

Salix Bebbiana Sargent. Rochers calcaires, talus argilo-calcaires. Rivières Vauréal (10 milles de la mer), au Saumon (10 milles de la mer), Sainte-Marie, Chicotte.

Salix Bebbiana Sargent var. *perrostrata* (Rydb.) Schneider. Berge argilo-calcaire. Rivière Sainte-Marie.

Salix brachycarpa Nutt. var. *Sansoni* Ball. Platières, berge graveleuse. Lac Wickenden, rivière Jupiter.

Salix candida Flugge. Tourbières, talus argilo-calcaires, tables calcaires, Hauteur des terres à la source des rivières au Saumon et Vauréal, ruisseau Cailloux, rivières à la Loutre, à la Chaloupe,

Salix candida Flugge f. *denudata* (Anderss.) Rouleau. Talus argilo-calcaires. Entre baie de l'Est et rivière Jupiter, rivière à la Chute.

Salix humilis Marsh. Berge argilo-calcaire. Rivière Sainte-Marie.

Salix myrtillifolia Anderss. Tables calcaires. Ruisseau Cailloux.

Salix pellita Anderss. f. *psila* Schneid. Platières graveleuses. Crique à la Chaloupe (7 milles de la mer).

URTICACÉES

Urtica gracilis Ait. Berge argilo-calcaire. Rivière Sainte-Marie.

SANTALACÉES

Comandra Richardsiana Fern. Prairie littorale. Rivière Jupiter.

POLYGONACÉES

Rumex Acetosella L. Grève. Crique à la Chaloupe.

Rumex Acetosella L. var. *integrifolius* Wallr. Tourbière. Rivière à la Loutre.

Rumex occidentalis S. Wats. Anse sablonneuse, berge argilo-calcaire. Ruisseau Bilodeau, anse du Morceau de Bois (à l'ouest de rivière Chicotte).

Polygonum viviparum L. Berge humide, platières. Anse aux Fraises, Port-Menier, lac Wickenden, rivières Jupiter, Vauréal (10 et 23 milles de la mer), au Saumon (43 milles de la mer), à la Chaloupe (7 milles de la mer).

Polygonum viviparum L. var. *pseudobistorta* Rousseau (55). Talus calcaires A l'est du ruisseau Cailloux.

CHENOPODIACÉES

Chenopodium album L. Ballast. Port-Menier.

Atriplex glabriuscula Edm. var. *oblanceolata* Vict. & Rousseau (47). Ligne de rivage. Port-Menier.

CARYOPHYLLACÉES

Cerastium vulgatum L. Berge. Rivière Sainte-Marie.

Stellaria calycantha (Ledeb.) Bongard. var. *laurentiana* Fern. Anfractuosités de rocher. Tête-à-la-vache (2 mi. à l'est de la rivière Galiote).

Stellaria crassifolia Ehrh. Affleurements calcaires. Ruisseau Cailloux.

Stellaria longipes Goldie. Orée du bois, sur la berge. Ruisseau Cailloux, rivière à la Chute.

Stellaria media (L.) Cyrillo. Berge graveleuse, prairies littorales. Rivières Saint-Marie, à la Chaloupe.

Sagina nodosa (L.) Fenzl. Table calcaire. Ruisseau Cailloux.

Arenaria lateriflora L. Grève. Rivière à la Chute.

RENONCULACÉES

Caltha palustris L. Platières herbeuses. Rivières Vauréal, à 8–9 milles de la mer.

Ranunculus abortivus L. var. *acrolasius* Fern. Bois clair, orée du bois. Rivières à l'Huile, McKane.

Ranunculus Purshii Richards. Rivière Sainte-Marie.

Ranunculus repens L. Ballast. Port-Menier.

Aquilegia vulgaris L. Fox bay. Introduit.

Coptis groenlandica Oeder. Berge argilo-calcaire. Lac Wickenden.

Anemone multifida Poir. var. *hudsoniana* DC. Talus graveleux, rivages calcaires. Rivières Vauréal (7 milles de la mer), au Saumon, (à 10 et 43 milles de la mer).

Anemone multifida Poir. var. *Richardsiana* Fern. Platières. Rivière à l'Huile.

Anemone parviflora Michx. Ayant parfois des fleurs d'au moins un pouce de diamètre. Tables calcaires, rivages graveleux. Rivières Jupiter, à l'Huile, Vauréal (7 et 23 milles de la mer), au Saumon, aux Plats, ruisseau Cailloux.

Anemone virginiana L. Corniches calcaires, Rivière au Saumon, à dix milles de la mer.

Thalictrum alpinum L. Berge graveleuse, ruisseaux ombragés. Lac Wickenden, rivière Sainte-Marie, ruisseau Cailloux, ruisseau Poulin, Rivières Jupiter, à la Chaloupe (7 milles de la mer), Vauréal (9 et 23 milles de la mer), au Saumon (43 milles de la mer).

Thalictrum polygamum Muhl. Platière herbeuse. Rivière au Saumon (35 milles de la mer).

Nymphéacées

Nuphar rubrodiscum Morong. Rivière Sainte-Marie.

Nuphar variegatum Engelm. Tourbières. Hauteur des terres, à la source des rivières Vauréal, au Saumon et à la Chaloupe.

Sarracéniacées

Sarracenia purpurea L. Berge tourbeuse, tourbières. Rivières Jupiter (embouchure), à la Loutre, Vauréal (9 milles de la mer). Hauteur des terres entre source des rivières Vauréal, au Saumon et à la Chaloupe.

Crucifères

Cakile edentula (Bigel) Hook. Ballast, Port-Menier.

Lepidium densiflorum Schrad. Ballast. Port-Menier.

Lesquerella Purshii Fern. Platières. Rivières Jupiter, Chicotte, Galiote.

Braya humilis (C. A. Meyer) Robinson. Platières, rivières Jupiter, Galiote (1).

Draba arabisans Michx. Grève. Rivière Sainte-Marie.

Draba glabella Pursh. Grève. Rivière Sainte-Marie.

Draba incana L. (*D. incana* var. *confusa* Poir.). Prairie littorale. Rivières à la Loutre, à la Chaloupe, Port-Menier, ruisseau Bilodeau.

Draba laurentiana Fern. Ligne littorale, talus argilo-calcaires. Rivières à la Loutre, au Saumon (à dix milles de la mer), ruisseau Cailloux.

Cardamine pennsylvanica Muhl. Rivière Sainte-Marie, en partie submergé.

Erysimum cheiranthoides L. Ballast, gravier littoral. Port-Menier, embouchure du crique à la Chaloupe.

Droséracées

Drosera anglica Huds. Tourbières. Source des rivières Vauréal, au Saumon et à la Chaloupe.

Drosera intermedia Hayne. Tourbière. Source de la rivière Vauréal (25 milles de la mer).

Drosera rotundifolia L. Tourbières. Rivière Vauréal (9 et 23 milles). Aussi berge argilo-calcaire. Ruisseau Bilodeau.

Violacées

Viola affinis LeConte var. *subarctica* Rousseau.* Berges humides, platières. Anse-aux-fraises, rivières Vauréal (8–9 milles de la mer), à la Loutre.

Viola nephrophylla Greene. Berges graveleuses. Lac Wickenden, rivière Sainte-Marie.

Viola pallens (Banks) Brainerd. Platières, tables calcaires. Rivières Galiote, Chicotte.

Saxifragacées

Saxifraga aizoides L. Platières, talus argilo-calcaires. Rivières à l'Huile, Vauréal (7 milles de la mer), Jupiter (embouchure), Pavillon, Chicotte.

Saxifraga oppositifolia L. Platières. Embouchure de la Jupiter.

Parnassia caroliniana Michx. Berge. Lac Wickenden.

Parnassia caroliniana Michx. f. *reducta* Rousseau.* Tables calcaires, platières. Rivières Jupiter, Cailloux, Vauréal (23 milles de la mer).

Parnassia parviflora DC. Corniches calcaires, talus argilo-calcaires, prairies littorales tourbeuses. Rivières à l'Huile, Sainte-Marie, Chicotte, à la Chaloupe (embouchure).

Mitella nuda L. Berge calcaire. Lac Wickenden.

Rosacées

Prunus depressa Pursh. Talus argilo-calcaires. Rivière au Saumon (dix milles de la mer).

Rosa johannensis Fern. Talus calcaires. Rivière Vauréal (7 milles de la mer).

Rubus acaulis Michx. Prairie tourbeuse. Crique à la Chaloupe (embouchure).

Rubus Chamaemorus L. Tourbière, berge argilo-calcaire. Crique à la Chaloupe, ruisseau Bilodeau. Vauréal (9 milles de la mer), hauteur des terres à la source des rivières Vauréal, au Saumon et à la Chaloupe.

Potentilla Anserina L. Berge. Port-Menier.

Potentilla Egedii Wormskj. var. *groenlandica* (Tratt.) Polunin. Prairies tourbeuses, platières graveleuses. Rivières Galiote, à la Chaloupe. Les

* *Pour description, voir supra.*

plantes des habitats naturels, sur Anticosti, appartiennent à cette espèce qui descend au sud jusque dans l'estuaire. (Fide Raymond.) Le *P. Anserina* est la plante introduite. Comme on le remarquera par cette liste, le quai de Port-Menier possède une collection très représentative des mauvaises herbes de la province.

Potentilla fruticosa L. Berges argilo-calcaires, platières. Lac Wickenden, rivières Chicotte, aux Plats, au Saumon (43 milles de la mer).

Potentilla tridentata Soland. Berges argilo-calcaires. Ruisseau Bilodeau.

Sanguisorba canadensis L. Berges argilo-calcaires, platières. Lac Wickenden, rivières Jupiter, Vauréal (23 milles de la mer), hauteur de terres à la source des rivières Vauréal, au Saumon et à la Chaloupe.

Dryas Drummondii Richards. Platières. Rivières McKane, Pavillon au Saumon (dix milles de la mer), à la Chaloupe (8–9 milles de la mer), Jupiter (partie inférieure), Chicotte, Galiote, Jupiter.

Dryas integrifolia Vahl. Platières. Rivières Galiote, Chicotte.

Légumineuses

Vicia Cracca L. Ballast. Port-Menier.

Lathyrus japonicus Willd. Ligne littorale. Port-Menier, rivière Jupiter (embouchure). Les spécimens de la rivière Jupiter portaient au collet, à la surface du sol ou sous un demi pouce de sable, des excroissances verdâtres, plus ou moins grosses, et atteignant parfois la taille de petites pommes de terre. Cette galle charnue est due à l'*Urophlictis Lathyri* (identification Emile Jacques). Cette galle avait été trouvée auparavant sur la même plante poussant sur la ligne littorale de Saint-Fabien, Co. Rimouski (Rousseau, 1936).

Lathyrus palustris L. var. *pilosus* (Cham.) Ledeb. Talus argileux. Rivière Jupiter.

Hedysarum alpinum L. var. *americanum* Michx. Corniches calcaires. Rivière au Saumon.

Trifolium hybridum L. var. *elegans* (Savi) Boissier. Voir Fernald (20). Phase extrême introduite mais non cultivée du *Trifolium hybridum* L. Sur le spécimen d'Anticosti, noter particulièrement les folioles à dents très aiguës, presque filiformes.

Trifolium repens L. Ballast. Port-Menier.

Onagracées

Circaea alpina L. Bois. Rivière à la Chute.

Epilobium angustifolium L. var. *macrophyllum* (Haussk.) Fern. Talus argilo-calcaires. Rivière au Saumon (10 milles de la mer).

Epilobium davuricum Fisch. Tourbières, rivière à la Loutre.

Epilobium glandulosum Lehm. var. *adenocaulon* (Haussk.) Fernald. Tables calcaires. Rivière Sainte-Marie.

Oenothera sp. Affleurements calcaires. Rivière Sainte-Marie.

Haloragidacées

Hippuris vulgaris L. et *Hippuris vulgaris* L. f. *fluviatilis* (Coss. & Germ.) Gluck. Rivière Sainte-Marie.

Myriophyllum Farwellii Morong. Rivière Sainte-Marie.

Acéracées

Acer spicatum Lam. Bois clair. Rivière à l'Huile.

Rhamnacées

Rhamnus alnifolia L'Hér. Berge graveleuse. Rivière Vauréal (21 milles de la mer).

Cornacées

Cornus canadensis L. Berge argilo-calcaire. Lac Wickenden.

Ombellifères

Sanicula marilandica L. var. *borealis* Fern. Platières, marge de la tourbière et du bois. Rivières Vauréal (embouchure et 7 milles de la mer),Galiote, Pavillon.

Ligusticum scothicum L. Ballast. Port-Menier.

Osmorhiza obtusata Fern. Berge humide. Anse aux Fraises.

Conioselinum chinense (L.) BSP. Orée du bois, berge. Lac Wickenden. Rivière Sainte-Marie.

Conioselinum chinense (L.) BSP var. *anticostense* Rousseau (55). Berge graveleuse. Lac Wickenden.

Conioselinum chinense (L.) BSP. var. *latilobum* Rousseau (55). Prairies littorales. Lac Salé.

Heracleum lanatum Michx. Tourbières au dessus de la berge, lieux humides. Port-Menier, rivières à la Loutre, Galiote. Très commun sur l'Ile.

Primulacées

Primula laurentiana Fern. Talus argilo-calcaires, tourbières littorales. Rivières à la Loutre, à la Chute, à la Chaloupe, Jupiter, Port-Menier.

Primula laurentiana Fern. f. *chlorophylla* Fern. Rochers calcaires, talus argilo-calcaires, prairies littorales, tourbières littorales. Rivières à la Loutre, Jupiter (embouchure), Galiote, à la Chaloupe (embouchure).

Primula mistassinica Michx. Talus argilo-calcaires, corniches calcaires, platières, gravier, Rivières Vauréal (7 et 23 milles de la mer), au Saumon (43 milles de la mer), Lac Wickenden, hauteur des terres à la source des rivières en Saumon et à la Chaloupe, rivières à la Loutre, Galiote, Chicotte.

Trientalis borealis Raf. Berge argilo-calcaire. Lac Wickenden.

Ericacées

Pyrola asarifolia Michx. var. *incarnata* (Fisch.) Fern. (Syn. *Pyrola uliginosa* Torr.). Platières. Rivières Vauréal (8–9 milles de la mer), aux Plats, Pavillon.

Pyrola virens Schweigg. (Syn. *P. chlorantha* Sw.) Talus argilo-calcaires. Rivière au Saumon (dix milles de la mer).

Ledum groenlandicum Oeder. Berge argilo-calcaire, tourbières. Lac Wickenden, rivière Vauréal (9 milles de la mer), hauteur des terres à la source des rivières Vauréal, au Saumon et à la Chaloupe.

Vaccinium myrtilloides Michx. (*V. canadense* Kalm). Rivages calcaires, bois de seconde venue. Rivières au Saumon (44 milles de la mer), à la Chaloupe (embouchure).

Vaccinium Oxycoccos L. Tourbières, berges argilo-calcaires. Ruisseau Bilodeau. Hauteur des terres entre sources des rivières Vauréal. au Saumon et à la Chaloupe.

Vaccinium uliginosum L. var. *alpinum* Bigel. Tables calcaires, platières. Ruisseau Cailloux, rivière Jupiter.

Vaccinium uliginosum L. var. *pubescens* Lange. Tourbières, bois de seconde venue. Rivière à la Chaloupe (embouchure).

Vaccinium Vitis-Idaea L. var. *minus* Lodd. Berges argilo-calcaires, tourbières. Lac Wickenden, rivière Vauréal (9 milles de la mer).

Epigaea repens L. var. *glabrifolia* Fern. Berge graveleuse. Lac Wickenden.

Arctostaphylos rubra (Rehder et Wilson) Fern. Platières. Embouchure de Jupiter.

Arctostaphylos Uva-ursi (L.) Spreng. var. *coactilis* Fern & Macbride. Berges graveleuses talus. Rivières Vauréal (21 milles de la mer), au Saumon (10 milles de la mer), à la Chaloupe (8–9 milles de la mer).

Chamaedaphne calyculata (L.) Moench. Tourbières. Hauteur de terre entre rivières Vauréal, au Saumon et à la Chaloupe.

Andromeda glaucophylla L. Tourbières, corniches calcaires. Rivières Vauréal (9 et 21 milles de la mer), Chicotte, hauteur des terres entre rivières Vauréal, au Saumon et à la Chaloupe.

Empétracées

Empetrum nigrum L. Au-dessus de la berge, au bord de la mer, tourbières. Rivière Sainte-Marie, hauteur des terres entre la source des rivières Vauréal, au Saumon, à la Chaloupe. Très commun partout.

Boraginacées

Mertensia maritima L. Ballast. Port-Menier.

Scrofulariacées

Pedicularis palustris L. f. *laurentiana* Vict. & Rousseau (47). Tourbière. Port-Menier.

Rhinanthus borealis Chabert. Prairie littorale. Crique à la Chaloupe.

Rhinanthus Kyrollae Chabert. Berge argilo-calcaire. Rivière Sainte-Marie.

Rhinanthus oblongifolius Fern. Prairie tourbeuse littorale. Rivière à la Loutre.

Castilleja septentrionalis Lindl. Platières, talus argilo-calcaires, prairies littorales. Rivières aux Plats, Jupiter, Pavillon, Galiote, Vauréal (7 milles de la mer), au Saumon (43 milles de la mer), lac Wickenden.

Melampyrum lineare Lam. Berge argilo-calcaire. Lac Wickenden.

Euphrasia arctica Lange. Partie supérieure de la grève halophytique et platières. Rivières Jupiter, à la Chute, Galiote.

Euphrasia hudsoniana Fern. & Wieg. Talus graveleux avec *Gentiana nesophila*. Entre rivière à la Loutre et ruisseau Cailloux.

Euphrasia Randii Robinson var. *Farlowii* Robinson. Platières, talus graveleux, portage au-dessus de la berge. Rivière Sainte-Marie, ruisseau Cailloux, rivière Jupiter.

Euphrasia stricta Host. var. *tatarica* (Fischer) Fern. & Wieg. Prairie fauchée, au-dessus du niveau de la mer. Port-Menier.

Lentibulariacées

Utricularia intermedia Hayne. Tourbière. Rivière Vauréal (9 milles de la mer).

Pinguicula vulgaris L. Platières, berges argilo-calcaires, tourbières. Rivières Jupiter, Vauréal (23 milles de la mer), au Saumon (43 milles de la mer), à la Chaloupe (7 milles de la mer), hauteur des terres à la source des rivières Vauréal, au Saumon, à la Chaloupe, ruisseau Bilodeau.

Orobanchacées

Orobanche uniflora L. var. *terrae-novae* (Fernald) Achey (2). Table calcaire. Ruisseau Poulin.

Labiées

Prunella vulgaris L. var. *lanceolata* (Barton) Fern. Platières. Talus argilo-calcaires. Rivières à l'Huile, Vauréal (23 milles de la mer), au Saumon (10 et 43 milles de la mer), Jupiter (6 milles de la mer).

Gentianacées

Menyanthes trifoliata L. var. *minor* Raf. Tourbières. Rivière Vauréal (9 milles de la mer), hauteur des terres à la source des rivières Vauréal, au Saumon, à la Chaloupe.

Gentiana acuta Michx. Prairie fauchée au-dessus du niveau de la mer. Port-Menier.

Gentiana nesophila Holm. Talus argilo-calcaires et affleurements calcaires. Est de la Rivière Sainte-Marie, ruisseau Cailloux, ouest de rivière Jupiter.

Lomatogonium rotatum (L.) Fries f. *americanum* (Griseb). Fernald. Tables calcaires. Rivières Sainte-Marie, Cailloux.

Lomatogonium rotatum (L.) Fries f. *tenuifolium* (Griseb.) Fernald. Prairie littorale. Rivière Jupiter.

Apocynacées

Apocynum androsaemifolium L. Talus argilo-calcaires. Rivières Jupiter (six milles de la mer), Vauréal (17 milles de la mer).

Oléacées

Fraxinus nigra Marsh. Platières graveleuses. Rivière Vauréal, embouchure et près de la chute.

Rubiacées

Galium Brandegeei Gray. Zone littorale. Rivière à la Chute.

Galium Claytoni Michx. Berge. Rivière Sainte-Marie.

Galium labradoricum Wiegand. Prairies de grève et platières. Rivières Vauréal et au Saumon, lac Wickenden.

Galium palustre L. Platières. Rivière Pavillon.

Galium tinctorium L. Platières. Rivière aux Plats.

Galium triflorum Michx. Grève. Lac Wickenden.

Caprifoliacées

Linnaea borealis L. var. *americana* (Forbes) Rehder. Berge argilo-calcaire. Lac Wickenden, rivière Vauréal (7 milles de la mer).

Viburnum cassinoides L. Berge graveleuse. Lac Wickenden.

Viburnum edule Raf. (syn. *V. pauciflorum* La Pylaie). Berge argilo-calcaire. Rivières Vauréal (23 milles de la mer), au Saumon (23 et 43 milles de la mer).

Diervilla Lonicera Mill. Talus calcaires. Rivière au Saumon (10 milles de la mer).

Lonicera villosa (Michx) R. & S. var. *Solonis* (Eaton) Fern. Tourbière. Rivière à la Chaloupe.

Campanulacées

Campanula rotundifolia L. Berges, platières. Rivières Sainte-Marie, à la Chute, aux Plats, Vauréal (7 et 23 milles de la mer), à la Chaloupe (7 milles de la mer), au Saumon (43 milles de la mer).

Campanula rotundifolia L. var. *alaskana* A. Gray. Prairie littorale. Rivière Jupiter. Déjà récolté à l'île Sainte-Paul, N.E. (Perry et Roscoe).

Campanula rotundifolia L. f. *albiflora* (G. Don) House. Berge. Rivière Sainte-Marie.

Lobéliacées

Lobelia Kalmii L. Berge graveleuse. Lac Wickenden.

Lobelia Kalmii L. f. *albiflora* Rouleau. Avec le précédent.

Composées

Taraxacum ceratophorum (Ledeb.) DC. (Mais plus probablement var. n. ou sp. n. Feuilles étroites, grosses achaines, tubercules des achaines recourbés). Talus argilo-calcaires. Rivières au Saumon (10 milles de la mer). *Rousseau 52233.*

Taraxacum lapponicum Kihlm. Platières et berges graveleuses, corniches calcaires, talus argilo-calcaires. Rivières à l'Huile, Vauréal (21 milles de la mer), au Saumon (à 10, 15, 25, 37 milles de la mer), à la Chute, aux Plats, Pavillon, à la Chaloupe (9 milles de la mer).

Taraxacum officinale Weber. Talus argilo-calcaires, berges graveleuses. Affleurements calcaires. Rivière Sainte-Marie, entre baie de l'est et rivière Jupiter.

Prenanthes altissima L. Affleurements calcaires. Ruisseau Cailloux.

Prenanthes altissima L. f. *integra* Rousseau (55). Grève, Lac Wickenden.

Prenanthes racemosa Michx. Platières. Rivière Jupiter.

Sonchus asper (L.) Mill. Ballast. Port-Menier.

Sonchus oleraceus L. Ballast. Port-Menier.

Hieracium canadense Michx. Corniches calcaires. Rivière au Saumon (10 milles de la mer). Le spécimen a une pubescence blanche cendrée peut-être due à l'état juvénile de la plante.

Hieracium groenlandicum Arv.-Thouv. Talus et falaises calcaires. Rivières Vauréal (en amont de la chute), au Saumon (à la source), Chicotte.

Tanacetum huronense Nutt. var. *bifarium* Fernald. Platières. Rivière Jupiter.

Tanacetum huronense Nutt. var. *bifarium* Fernald f. *lanatum* Rousseau.* Platières. Rivière McKane.

Tanacetum huronense Nutt. var. *terrae-novae* Fernald. Platières. Rivière Pavillon.

Artemisia biennis Willd. Ballast. Port-Menier.

Artemisia borealis Pallas f. *typica*. Platières. Rivière Jupiter.

Artemisia canadensis Michx. f. *rupestris* Vict. & Rousseau (48). Platières. Rivière Jupiter.

Antennaria canadensis Greene. Berges calcaires, platières graveleuses. Rivières Vauréal (à 17 milles de la mer), rivière Pavillon.

Antennaria gaspensis Fern. Talus calcaires ou argilo-calcaires, et platières. Rivières Vauréal (à l'embouchure et 7 milles de la mer), au Saumon (10 et 15 milles de la mer), à la Chute.

* *Pour description, voir supra.*

Antennaria neodioica Greene var. *chlorophylla* Fern. Tables calcaires inondées au printemps. Rivière au Saumon (38 milles de la mer), source du ruisseau Poulin.

Antennaria pulcherrima (Hook.) Greene. Platières. Rivières Jupiter, Pavillon, Galiote.

Antennaria spathulata Fern. Berge et platières graveleuses. Rivières à l'Huile, Vauréal (embouchure), à la Chaloupe.

Anaphalis margaritacea (L.) Gray var. *subalpina* Gray. Grève. Lac Wickenden.

Gnaphalium sylvaticum L. Bois clair. Rivière Sainte-Marie.

Petasites palmatus (Ait.) Gray. Bois, berge calcaire, talus argilo-calcaire grèves graveleuses. Lac Wickenden, rivières à l'Huile, Vauréal (17 et 23 milles de la mer), au Saumon (43 milles de la mer), à la Loutre, Jupiter (6 milles de la mer).

Petasites vitifolius Greene. Prairie herbeuse littorale. Tête-à-la-vache (2 milles à l'est de la rivière Galiote).

Senecio aureus L. (var. non identifiée). Berge du lac Wickenden, rivière Sainte-Marie.

Senecio aureus L. var. *aquilonius* Fern. Berges et platières graveleuses. Rivières à l'Huile, Vauréal (à 21 milles de la mer).

Senecio aureus L. var. *intercursus* Fern. Platières graveleuses. Rivière Jupiter.

Senecio gaspensis Greenm. Platières et berges graveleuses, tables calcaires. Rivières à l'Huile, Vauréal (à 21 milles de la mer), ruisseaux Cailloux, rivières Jupiter (embouchure) à la Chaloupe (7 milles de la mer).

Senecio gaspensis Greenm. var. *firmifolius* (Greenm.) Fern. Berges et platières graveleuses, talus calcaires, prairies littorales. Lac Wickenden, rivières à l'Huile, Vauréal (7 milles de la mer), ruisseau Poulin, rivières Jupiter (embouchure), Pavillon.

Senecio gaspensis Greenm. var. *Victorini* Rousseau.* Berge argilo-calcaire. Rivière Chicotte.

Senecio pauperculus Michx. Typique. Corniches calcaires, talus argilo-calcaires, tourbière sèche. Lac Wickenden, rivières au Saumon (10 milles de la mer), Chicotte.

Senecio pseudo-Arnica Less. Ligne littorale. Pointe du Sud-ouest.

Cirsium muticum Michx. Platières. Rivière Chicotte.

Chrysanthemum Leucanthemum L. var. *pinnatifidum* Lecoq & Lamotte. Ballast, Port-Menier.

Achillea lanulosa Nutt. Talus calcaire. Rivière Vauréal (7 milles de la mer).

Achillea Millefolium L. Ballast. Port-Menier.

* *Pour description, voir supra.*

Arnica chionopappa Fern. Falaises et talus calcaires. Rivières Vauréal (7 et 10 milles de la mer), au Saumon (10 milles de la mer), Chicotte.

Solidago anticostensis Fernald. Talus argilo-calcaire. Ruisseau McGilvray.

Solidago chlorolepis Fernald. Platières. Rivière Jupiter (embouchure).

Solidago graminifolia (L.) Salisb. var. *Grahami* Rousseau (55). Berge argilo-calcaire. Ruisseau McGilvray, pointe du Sud-ouest. Le P. Bernard Taché a rapporté le 8 août 1944 de la pointe du Sud-ouest deux spécimens (Nos 44131 et 44140) qu'il faut rattacher à cette variété, mais contrairement au spécimen-type, ils ont un feuillage compact formant une touffe dense. Les spécimens du ruisseau McGilvray, au contrairie, avaient un feuillage étalé.

Solidago hispida Mühl. Berges et platières graveleuses, talus argilo-calcaires, tables calcaires. Rivières Vauréal (6 et 7 milles de la mer), au Saumon (10 et 43 milles de la mer), Sainte-Marie, McKane, Pavillon, Chicotte. L'une des verges d'or les plus fréquemment récoltées. Très polymorphe, il faudrait en faire l'étude sur une autre base que la pubescence du feuillage (tout au plus utile pour déterminer les formes secondaires chez cette espèce). La forme des bractées involucrales (aiguës lancéolées, ovées-obtuses) semble pouvoir fournir de bons caractères.

Solidago hispida Mühl. var. *arnoglossa* Fernald. C'est à cette variété qu'il faut rattacher, semble-t-il, le spécimen suivant. Rivière Jupiter, platière herbeuse à l'embouchure, 21 juillet 1942. *Rousseau 52407A*. La récolte ne comprend malheureusement qu'un spécimen qui n'est pas encore à maturité complète.

Solidago racemosa Greene. Les plantes d'Anticosti toutefois ne correspondent pas très bien à la description de Greene. Pour discussion, voir plus haut la description de ×*Solidago Raymondi*. Platières, corniches calcaires, talus argilo-calcaires. Rivières Jupiter (embouchure et à 6 milles de la mer), Chicotte.

×*Solidago Raymondi* Rousseau.* Platières et berges graveleuses. Rivières Pavillon, Chicotte, Jupiter.

Solidago uliginosa Nutt. var. *linoides* (Torr. & Gray) Fernald. Berge graveleuse, corniches calcaires, tourbières. Rivières au Saumon (25 milles de la mer), à la Chaloupe (15 milles de la mer), à la Loutre.

Solidago Victorinii Fern. Platières et berges graveleuses. Rivières Pavillon, Chicotte.

Erigeron hyssopifolius Michx. Platières, tables calcaires, prairies littorales. Ruisseau Cailloux, rivières Jupiter, à l'Huile, Galiote, aux Plats, Vauréal (23 milles de la mer), au Saumon (43 milles de la mer).

Erigeron lonchophyllus Hook. var. *laurentianus* Vict. (37). Ligne littorale, protégée par banc graveleux. Rivière Jupiter.

* *Pour description, voir supra.*

Aster anticostensis Fern. Talus argilo-calcaires. Entre baie de l'Est et rivière Jupiter.

Aster foliaceus Lindl. var. *arcuans* Fernald. Berge graveleuse. Lac Wickenden.

Aster foliaceus Lindl. var. *subpetiolatus* Fernald. Talus calcaires. Entre rivière à la Loutre et ruisseau Cailloux.

Aster ciliolatus Lindley (syn. *A. Lindleyanus* T. & G.). Orée du bois. Lac Wickenden.

Aster ciliolatus Lindley var. *borealis* (Rousseau), Dutilly et Lepage (10). Grève. Lac Wickenden [Primitivement décrit comme *Aster Lindleyanus* T. & G. var. *borealis* Rousseau (55).].

Aster longifolius Lam. Tourbière. Lac Wickenden.

Aster novi-belgii L. var. *elodes* (T. & G.) Gray. Platières. Rivière Jupiter.

Aster novi-belgii L. var. *rosaceus* Rousseau (55). Talus argilo-calcaires. Rivières Sainte-Marie et Jupiter, ruisseau Cailloux.

Aster puniceus L. var. *oligocephalus* Fern. Talus argilo-calcaires. Entre baie de l'Est et Jupiter.

Aster Radula Ait. Grève. Lac Wickenden.

Aster tardiflorus L. Berge. Rivière Sainte-Marie.

Aspects phytogéographiques

La flore d'Anticosti pose de nombreux problèmes phytogéographiques. Dans la présente étude, il ne sera question que des affinités arctiques et cordillériennes. Par contre, Marcel Raymond, dans une étude des *Carex*, qui paraîtra incessamment, en aborde plusieurs autres. Il est juste de noter qu'il n'y a sûrement pas de genre dans le nord de l'amérique plus propre à une étude phytogéographique que celui des *Carex*. Sous toutes les latitudes, de l'est à l'ouest, et dans tous les habitats, poussent de nombreux *Carex*. Genre extrêmement riche, ses espèces,—contrairement à beaucoup d'*Antennaria*, de *Potentilla*, d'*Astragalus*, de *Taraxacum* et d'autres,— ne peuvent être considérés comme des jordanons. Malgré leurs problèmes taxonomiques, bien rares sont les *Carex* sur lesquels ne s'entendent pas la plupart des systématistes. En phytogéographie et en écologie, les *Carex* pourraient constituer probablement le genre-clef.

Tous les travaux du F. Marie-Victorin sur la flore de la région du golfe Saint-Laurent renferment des aspects phytogéographiques. Tous contribuent à l'édification de ses hypothèses sur la flore allogène d'Anticosti-Minganie, qui trouvent leur forme la plus définitive dans la *Flore Laurentienne* (44) et dans *Phytogeographical Problems of Eastern Canada* (46).

Dans la *Flore Laurentienne* (44) au chapitre remarquable sur "la carte phytogéographique du Québec", la région qui nous intéresse actuellement fait

l'objet de la "section de l'avant-pays (Anticosti-Minganie)". Dans un exposé succinct des caractéristiques générales, le F. Marie-Victorin écrit d'abord:—

> "Si l'on fait abstraction des facteurs historiques, liés à l'évolution organique et aux vicissitudes géologiques de l'Amérique orientale, le caractère général de la végétation de l'Anticosti-Minganie ressortit à deux séries de facteurs principaux. En premier lieu, la latitude élevée, la faible altitude et le voisinage des courants froids imposent la végétation subarctique propre à la partie sud du Labrador canadien. En second lieu, la nature entièrement calcaire des roches horizontales stratifiées qui constituent l'Anticosti-Minganie exerce une influence profonde sur la structure de la flore et sur le choix des espèces.
>
> Ces facteurs, agissant simultanément sur tous les points d'un territoire essentiellement isomorphe, devraient lui constituer une flore uniforme, sans autres différenciations que celles qui sont engendrées par les nécessités écologiques locales et les différences d'habitat. Cette vue théorique n'est aucunement chimérique: elle trouve sa réalisation adéquate dans la vaste étendue des Laurentides où la flore, dénuée d'endémiques vrais ou d'épibiotes, répète partout les mêmes facies et les mêmes associations. Elle se concrétise encore dans toute la partie centrale de l'Ungava, dans la toundra arctique, et, abstraction faite de la Minganie et des abords du détroit de Belle-Isle, sur toute la Côte-Nord."

Une connaissance plus étendue de la flore du Québec, due à des explorations postérieures dans l'Abitibi (région de Duparquet, Rousseau 1938), au lac Mistassini (Lepage et Dutilly (10), Rousseau et Rouleau, entre 1943 et 1947), et dans l'intérieur de l'Ungava (Lepage, Dutilly, Rousseau, 1944, 1947, 1948 et 1949) ne permettrait plus aujourd'hui d'accepter cette opinion. Le sujet est traité de façon plus élaborée dans deux autres études sous presse (57, 58). Pour le problème d'Anticosti, seules les notions acquises au cours des voyages de 1940 et 1942 feront l'objet de discussion.

Après des notions générales sur la Flore d'Anticosti, le F. Marie-Victorin mentionne les endémiques stricts du territoire (dont l'un, le *Cirsium minganense*, est nettement d'affinité cordillérienne), les espèces arctiques ou alpines, enfin les espèces cordillériennes et les endémiques d'affinité cordillérienne. Puis l'auteur conclut:

> "De longs et minutieux travaux sur le terrain ont mis en évidence plusieurs faits importants concernant l'Anticosti-Minganie. La masse des entités endémiques ou reliquales est localisée sur le littoral des îles de la Minganie, et pour l'île d'Anticosti, sur les graviers des rivières ou des terrasses marines, mais presque exclusivement du côté sud. De plus, un pointage sur la carte montre que ces éléments floristiques allogènes sont répartis sur une bande nord-sud qui exclut les extrémités de l'île d'Anticosti (Carte J). Les faits biologiques semblent indiquer que cette bande, malgré son peu d'altitude, est un nunatak, probablement un sinus entre deux champs glaciaires. La conservation de cette florule ancienne, héritée de la dernière époque interglaciaire, exige aussi que les îles de la Minganie et l'île d'Anticosti, n'aient pas été complètement submergées durant la période Champlain et se soient trouvées surélevées à cette époque. Il y a lieu de croire que, pour Anticosti en particulier, le modeste plateau de l'intérieur, drainé par les rivières Jupiter, Galiote, Chicotte, etc., émergeait à l'état d'îlot à la période Champlain. Couvert d'une végétation rabougrie, il devait servir de refuge aux plantes que nous considérons comme des épibiotes et que nous retrouvons aujourd'hui sur les graviers des rivières. Cette partie élevée de l'île est maintenant complètement boisée et les reliques préglaciaires, plantes de prairies alpines exigeant la pleine lumière, se sont réfugiés dans le seul habitat qui pouvait leur permettre de subsister: les platières graveleuses, éclairées, froides, des petites rivières descendant du plateau central de l'intérieur (Carte K). Il est possible que cette bande de l'Anticosti-Minganie soit continue avec celle qui renferme le territoire à reliques et à endémiques de la Gaspésie."

Dans une étude ultérieure, Wynne-Edwards (65) prétend démontrer que plantes cordillériennes et arctiques ne sont qu'une seule et même chose:

> "No distinction is possible between cordilleran and arctic species when their ranges are examined. The details of distribution of one group may be paralleled by those of the other. The northern flora may be roughly segregated into holarctic and nearctic types, but in both groups some species are ubiquitous and others have disrupted ranges."

Impressionné par cette opinion, le F. Marie-Victorin propose (46) son hypothèse de l'arc-en ciel ("Rainbow explanation"):

> "Perhaps, one of the weakest points of Fernald's nunatak hypothesis, at least in the early statements, is the sharp distinction stressed or maintained between truly arctic and cordilleran plants, the latter in the sense of plants having migrated from the Cordillera eastward in preglacial or interglacial times. The abandonment of this distinction would perhaps clarify the situation and strengthen the case. May not all, or most, of the plants concerned, be considered as arcto-tertiary plants having migrated from their high northern station along the two main paths offered to them on either side of Hudson's Bay? May not most of the so-called Cordilleran plants be just arctic migrants that for some reason or another have become extinct in the Arctic and have persisted at one or both ends of their migrating trails? (Fig. 60)."

Tout le problème peut se ramener à trois questions: 1. Les plantes arctiques et les plantes cordillériennes sont-elles une seule et même chose? 2. L'hypothèse de la présence d'un refuge au centre d'Anticosti peut-elle se défendre à la lueur des connaissances actuelles? 3. Si Anticosti n'est pas un nunatak, comment expliquer sa florule allogène? On pourrait même se demander: En admettant, pour fins de discussion, qu'il n'y ait aucune raison sérieuse à invoquer contre l'hypothèse du nunatak d'Anticosti, est-ce la seule explication plausible?

1. *Plantes cordillériennes et plantes arctiques*

Certaines espèces vivent dans l'Arctique, non seulement parce que la saison de végétation y est courte, mais parce que la période diurne d'insolation l'été y est très longue. Ce sont les véritables plantes arctiques. D'autres vivront en montagne, bien au sud de l'Arctique, mais pas dans cette dernière zone, parce que si, d'une part, elles se contentent d'une courte saison de végétation et exigent la pleine lumière, elles ne tolèrent pas, d'autre part, une trop longue période diurne d'insolation, l'été. Ces plantes sont de véritables espèces alpines, et il y en a vraisemblablement de "réglées" pour des périodes diurnes de diverses longueurs. Parmi celles-ci se placeraient les espèces cordillériennes et gaspésiennes proprement dites. C'est ainsi que les véritables plantes alpines de la Gaspésie, où la journée de lumière est de seize à dix-huit heures l'été, n'ont rien de commun avec les plantes alpines du Mexique où les plus grandes journées de lumière ne dépassent pas sensiblement douze heures. Ceux qui ont essayé d'acclimater dans les jardins botaniques, sous la latitude de Montréal, par exemple, des espèces du Pérou savent les problèmes que cela pose. Au voisinage du névé sur le Popocatepelt, les espèces alpines ne rappellent pas celles des montagnes de la Gaspésie ou des territoires arctiques du Québec, où les conditions de température sont sensiblement équivalentes. Parmi nos plantes alpines proprement dites se placeraient les vraies plantes cordillériennes (ou gaspésiennes), par exemple, ***Polystichum mohrioides*** var.

scopulinum. Enfin, en dehors des espèces arctiques proprement dites et des espèces alpines proprement dites, il y a les espèces indifférentes arctiques-alpines.

Est-ce dire que toutes les espèces cordillériennes absentes de l'Arctique sont purement alpines. Pas nécessairement. Il est possible, et même probable, comme le croient Wynne-Edwards et le F. Marie-Victorin que des espèces aujourd'hui cordillériennes ont vécu déjà dans l'Arctique avant que divers facteurs ne les en éliminent. Il est possible aussi que des plantes, limitées aujourd'hui à l'Arctique, soient des espèces arctiques-alpines indifférentes ayant habité autrefois la Cordillère et les montagnes de la Gaspésie. Nos espèces arctiques-alpines sont les seules sur lesquelles on est parfaitement renseigné. Quant aux autres de notre territoire,—car pour celles des montagnes du sud de la Californie, du Mexique et du Pérou, le problème ne se pose presque plus,—il n'y a que les cultures expérimentales pour nous renseigner. C'est là une tâche pour les jardins botaniques.

Admettons pour les fins de la discussion,—ce qui paraît éminemment vraisemblable,—que les éléments allogènes d'Anticosti, comprennent à la fois des espèces arctiques proprement dites, des espèces alpines (cordillériennes) proprement dites et des espèces arctiques-alpines.

2. *Le refuge du centre d'Anticosti*

Après deux explorations du centre d'Anticosti,—point d'origine de la plupart des grandes rivières de l'Ile, où aurait pu se trouver le refuge sur lequel le F. Marie-Victorin fondait tant d'espoirs,—puis des reconnaissances du territoire en avion, il est évident qu'il faut renoncer définitivement à l'hypothèse du refuge central. Le centre est à peine plus élevé que la rive nord et occupé par des tourbières surélevées servant de réservoir aux rivières des deux rives. En outre, rien, dans l'état actuel de nos connaissances, ne permet de croire que l'Ile ait échappé à la submersion de la mer Champlain. Si elle héberge actuellement des espèces préglaciaires, il faut donc qu'ils viennent de nunataks extérieurs. Car à quoi bon pour Anticosti d'avoir été un nunatak si la submersion Champlain devait l'effacer de la carte.

3. *La théorie des nunataks et celle des avant-postes arctiques et alpins*

Il n'y a pas lieu d'aborder ici dans l'ensemble le problème des nunataks du nord-est de l'Amérique. Cela fait l'objet d'autres études. Que la Gaspésie ait subi ou non la glaciation importe peu dans le problème d'Anticosti, puisqu'il faut expliquer la colonisation de cette île par des épibiotes après la submersion Champlain.

Déjà des travaux sur la flore de l'estuaire, de la région de Saint-Urbain et de Matapédia et la monographie des *Astragalus* du Québec avaient permis à l'auteur d'expliquer la présence de plantes reliquales autour du golfe Saint-Laurent par la persistance d'îlots subarctiques (51, 52, 53). En effet, devant le glacier quaternaire se trouvait une bande de toundra et la vallée du Saint-Laurent fut forcément occupée par la toundra à "l'époque présylvatique" (53). Quand la forêt envahit cette bande, il persista néanmoins des habitats limités

arctiques ou subarctiques où les conditions adverses éliminaient les plantes méridionales plus agressives. Si les plantes de la zone tempérée ne peuvent vivre dans l'Arctique parce que la courte saison de végétation s'y oppose, par contre la plupart des plantes arctiques peuvent vivre dans la zone tempérée et même y constituer d'excellentes espèces de rocailles, pourvu qu'on élimine la concurrence. Sur les falaises et corniches exposées, comme celles du Bic et du cap des Rosiers, d'une part, sur la berge des cours d'eau et dans le lit des rivières, torrentielles au printemps, où émergent des grandes platières au cours de l'été, comme la Restigouche, la Matapédia et celles d'Anticosti, d'autre part, l'exposition aux intempéries dans le premier cas, la courte période d'émersion dans le second cas, ne permettant guère à la flore tempérée de s'établir à demeure, laissant ainsi le champ libre aux espèces arctiques et subarctiques.

Toutes les espèces arctiques et subarctiques de l'île Anticosti ont pu ainsi y émigrer par le corridor de toundra, en marge du glacier à l'époque présylvatique, et y être conservées depuis sur des parcelles demeurées arctiques ou subarctiques. Au nom de ces parcelles, désignées d'abord comme "îlots subarctiques" (53), il importe de substituer aujourd'hui celui plus correct d'**avant-postes arctiques** et d'**avant-postes subarctiques**, suivant le cas. Le mot "avant-poste", ajouté à l'épithète arctique ne risque pas, comme *îlot*, de prêter à confusion.

Quant aux véritables espèces alpines (cordillériennes) qui ont émigré dans la région du golfe Saint-Laurent sans pouvoir passer par l'Arctique, elles aussi ont pu suivre également le corridor désertique bordant le glacier. Des Montagnes Rocheuses au golfe Saint-Laurent, le jour-lumière était partout le même en marge du glacier. Les véritables plantes cordillériennes pouvaient trouver là les conditions nécessaires à leur migration: absence d'arbres, habitat désertique, jour-lumière de longueur définie. Tant que l'Amérique du Nord jouissait d'un climat tempéré, les plantes cordillériennes ne pouvaient émigrer de l'ouest à l'est, la barrière des prairies et de la forêt laurentienne s'y opposant; mais quand le glacier eut anéanti la forêt et donné à la lisière de continent qui le bordait un facies arctique-alpin, plusieurs espèces cordillériennes y trouvèrent le pont nécessaire à la migration. Ces espèces pouvaient avoir été protégées sur des nunataks de la Cordillère ou dans la partie de ces montagnes au sud de la limite extrême de l'avance du glacier, grosso modo au niveau d'une partie du territoire entourant le golfe Saint-Laurent. Cette migration a pu se faire pendant et après la glaciation Wisconsin, tant que la forêt ne vint pas occuper tout le territoire. Après cela, il ne restait plus pour faciliter la migration que la bordure de l'océan Arctique et le littoral de la baie d'Hudson et de la baie James. Les plantes cordillériennes abandonnées dans l'est ont pu persister là où les conditions rappelaient celles de l'habitat original: longueur de jour-lumière, absence d'arbres, conditions désertiques pendant la courte saison de végétation. Ces habitats restreints devraient se nommer **avant-postes alpins** ou **cordilléries.** Les avant-postes alpins et arctiques se superposent généralement, mais il y a néanmoins lieu de conserver

les deux notions, car l'expérimentation nous apprendra sûrement à distinguer ce qui est alpin de ce qui est arctique. Pour de nombreuses espèces, la distinction semble assez nette déjà.

Cette interprétation des avant-postes alpins et arctiques explique facilement la présence d'expèces reliquales sur un territoire ayant probablement subi la submersion Champlain, comme celui d'Anticosti-Minganie. Le même concept peut être appliqué sans doute à la Gaspésie, mais le problème de la présence ou de l'absence d'un nunatak en Gaspésie demande un exposé plus élaboré où il faudra faire intervenir les éléments fournis par les dernières explorations du lac Mistassini et de l'Ungava.

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SPIKE DENSITY, SPELTOIDY, AND COMPACTOIDY IN HEXAPLOID WHEAT[1]

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Abstract

F_2 progeny from F_1 plants monosomic for chromosome IX of Hymar obtained from the cross of chromosome IX deficient Chinese Spring × Hymar, a variety of club wheat, were studied for spike type segregation and chromosomal constitution. Spikes from plants with two members of chromosome IX from Hymar were club, intermediate, or common-type. Spikes from plants monosomic for chromosome IX were likewise club, intermediate, or common type but all had typical speltoid characteristics. When the spike type segregates were combined, there resulted an almost perfect fit to a 1 : 2 : 1 ratio of club, intermediate, and common spike type plants, respectively, indicating that chromosome IX was not associated with inheritance of spike type. It is suggested that subcompactoidy and compactoidy resulting from an increase in the dosage of chromosome IX is caused by an increase in the dosage of the gene for squareheadedness carried by this chromosome. The shortening of plants having higher dosages of chromosome IX is probably caused by abnormal chromosomal constitution resulting in genetic unbalance.

Li *et al.* (6) have proposed a simple hypothesis to account for the differences between three species of hexaploid wheat. They postulated that *Triticum spelta* has zero, *T. vulgare* two, and *T. compactum* four doses of what they term the speltoidy complex on the distal ends of the long arms of chromosome pair IX. The hypothesis was based on observations of a progressive change from speltoidy to compactoidy relative to the increase in dosage of the speltoidy complex of chromosome IX. The speltoidy complex, they believe, functions in inhibiting the formation of auxins which are directly responsible for the characteristic differences between the three species.

The validity of this hypothesis can be checked by crossing a plant of *T. vulgare* deficient for chromosome IX with a normal plant of *T. compactum*. According to the hypothesis the results should be as follows:

(1). The monosomic F_1 plants would all have one chromosome IX from *T. compactum*. These plants would all have two doses of the speltoidy complex and so all should have common (vulgare-like) spikes.

(2). F_2 plants with 21 pairs of chromosomes would have two chromosomes IX from *T. compactum*. They would have four doses of the speltoidy complex, and all should therefore have club (compactum-like) spikes.

(3). Monosomic F_2 plants would have the same speltoidy complex dosage as the F_1 and all should have common type spikes.

(4). F_2 plants nullisomic for chromosome IX would have zero dosage of the speltoidy complex, and all should be speltoid.

[1] *Manuscript received January 12, 1950.*

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In this paper the results of a study of the F_2 progeny from a cross involving chromosome IX deficient Chinese Spring with Hymar, a variety of *T. compactum* are presented and discussed.

Review of Literature

Speltoidy resulting from the loss of a C chromosome has been fully reviewed by Huskins (5). His conclusion, that increase in the dosage of the C chromosome, or an essential portion of it, resulted in subcompactoidy or compactoidy has been substantiated by the recent investigations of Akerman and MacKey (1), Sanchez-Monge and MacKey (8), and Li *et al.* (6). In only the latter study, however, is there an attempt to associate the compactoidy resulting from abnormal chromosome constitution with species differences. In numerous other investigations (11, 4, 3, 12, 13, 14, 2) of crosses between varieties of *T. vulgare* and *T. compactum* one main gene for spike density has been found to operate. However, in most cases transgressive segregation has been observed. Nilson-Ehle (7) suggested that this transgressive segregation was caused by genes promoting length of rachis internode, which were hypostatic to the gene for dense spikes. In crosses of 17 lines of Chinese Spring (each deficient for a different chromosome) with the club variety Hymar, Unrau (15) associated the gene for the dense spike of Hymar with chromosome XX. Segregation of spike type in chromosome IX did not differ from that found in other families. In a personal communication Dr. Sears has informed the senior author that "the gene for dense spikes in Red Egyptian and apparently in Poso is carried by chromosome XX". From these results chromosome IX does not necessarily appear to be associated with spike type as suggested by Li *et al.* (6).

Material and Methods

F_2 seed from the cross of chromosome IX deficient Chinese Spring × Hymar was available from a previous study (15). The seed was space planted in the greenhouse. At sporogenesis a spike from each plant was fixed in 6 : 3 : 1 Carnoy's fluid for cytological examination. At maturity the spike type of each plant was recorded.

Cytological examinations were made of pollen mother cells in acetocarmine smear preparations at a magnification of approximately 400 diameters. Photomicrographs were taken of slides made permanent by the tertiary butyl alcohol method as outlined by Smith (10).

Experimental Results

In Table I the chromosomal constitutions and spike types of 238 F_2 plants are summarized.

These results are very significant in the light of the hypothesis of Li *et al.* (6). In the group with 21 pairs of chromosomes, where in every case two IX chromosomes were present from Hymar, the *T. compactum* parent, there were compact, intermediate, and lax or common-type spikes (Fig. 1). If the

PLATE I

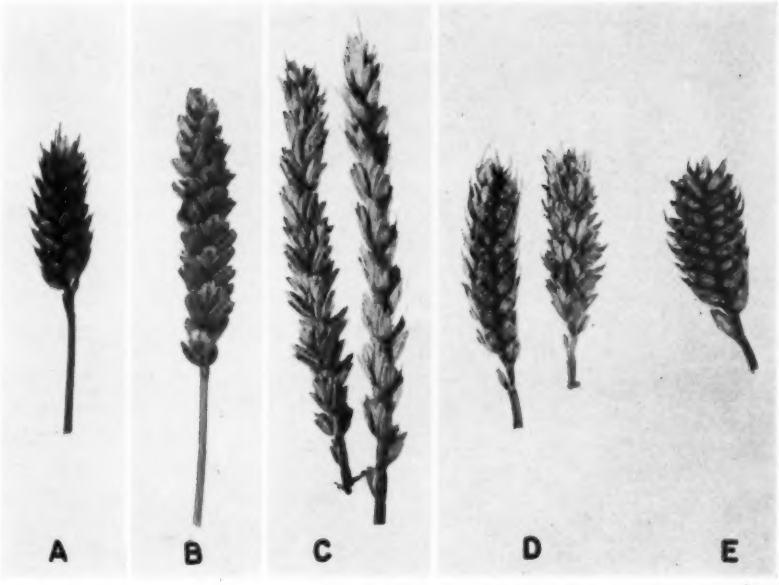

FIG. 1. *A—Hymar, B—normal Chinese Spring, C, D, and E—common, intermediate, and club type spikes, respectively, from F_2 plants with 21 pairs of chromosomes.*

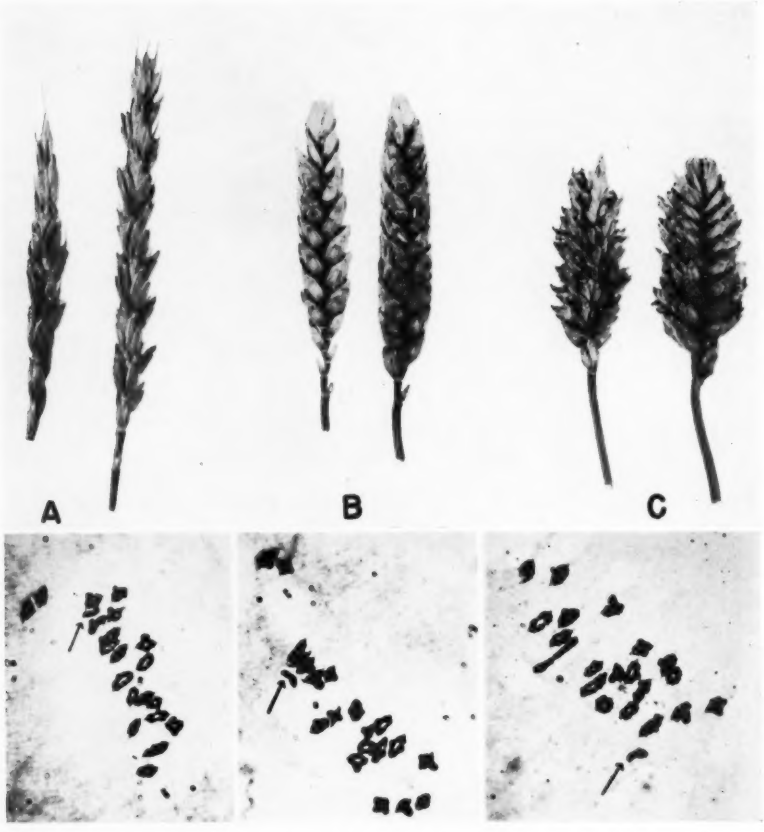

FIG. 2. *Spikes and sporocytes from F_2 plants of chromosome IX deficient Chinese Spring × Hymar.*

A—from plant with common type spikes.
B—from plant with intermediate spikes.
C—from plant with club type spikes.

(*Sporocytes are from the same plants as spikes, arrows indicate univalents.*)

TABLE I

CHROMOSOMAL CONSTITUTION AND SPIKE TYPE OF THE F_2 PROGENY FROM THE CROSS OF CHROMOSOME IX DEFICIENT CHINESE SPRING × HYMAR

Spike type	Chromosomal constitution		
	21^{II}	$20^{II}1^{I}$	Total
Club	27	30	57
Intermediate	25	96	121
Common	16	44	60
Total	68	170	238

hypothesis of genic dosage of the speltoidy complex were wholly correct, all F_2 plants with 21 normal pairs of chromosomes should have had club type spikes since they had four doses of the speltoidy complex.

In the group with the $20^{II}1^{I}$ constitution, likewise, there were plants with dense, intermediate, and common-type spikes. Since monosomic plants had the one chromosome IX from Hymar, they all should have had common-type spikes because they had two doses of the speltoidy complex. Regardless of spike density, monosomic plants had speltoid characteristics as illustrated in Fig. 2. This fact is important since it clearly indicates that speltoidy develops independently of spike density, whenever one chromosome IX is missing.

While no monosomic F_1 spikes were available when this study was initiated, they were similar to the monosomic F_2 spikes of intermediate density illustrated in Fig. 2B. They also exhibited typical speltoid spike and glume characteristics.

No nullisomic F_2 plants were found probably because the plants were grown under somewhat crowded conditions. If nullisomic seedlings were present, it is likely that they failed to survive.

It is significant that when the chromosomal constitution of the F_2 plants is disregarded, there results an almost perfect fit to a 1 : 2 : 1 ratio of dense, intermediate, and lax-spiked plants. This is to be expected if the inheritance of spike density is not associated with chromosome IX.

Discussion

The results of this study show that chromosome IX is not responsible for differentiation of the three hexaploid species with respect to spike density. As has been pointed out earlier (cf. Review of Literature), the gene for dense spikes is carried by chromosome XX in at least three varieties of *T. compactum*. It has further been shown in this study that speltoidy results when one chromosome IX is missing, regardless of the density of the spike.

Why then is there a change to compactoidy when the dosage of chromosome IX or the speltoidy complex portion is increased? And why is this accompanied by a general shortening of the chromosomally aberrant plants? The

authors suggest that the answer to the first question might be the result of action of the gene for squareheadedness known to be carried by chromosome IX (9). The action of this gene results in a shortening of the internodes at the apical end of the rachis. When the dosage of chromosome IX is increased by any one of the methods suggested by Li *et al.* (6) the dosage of the squarehead gene also would be increased. At higher dosages the action of this gene would result in subcompactoidy or even compactoidy. With respect to the general shortening observed in plants having higher dosages of chromosome IX, it is conceivable that this is the result of the aberrant chromosomal constitution of such plants.

Acknowledgments

The authors would like to express their thanks and appreciation to Dr. L. P. V. Johnson, Associate Professor of Genetics and Plant Breeding, University of Alberta, who has read and criticized the manuscript.

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TUMOR INDUCTION IN *VICIA FABA* AND OTHER HOSTS BY *AGROBACTERIUM RUBI* (HILDEBRAND) STARR AND WEISS[1]

By L. C. Coleman

Abstract

In a search for suitable material for a cytological study of plant tumors, pure and virulent cultures of *Agrobacterium tumefaciens* were inoculated into stems of young *Vicia faba* plants without result. On the other hand, a bacterium isolated from Himalaya blackberry produced large tumors. A comparison of the two forms as to their virulence on other plant species showed that the blackberry organism would not attack tomato, *Nicotiana glutinosa*, and *Datura medeloides*, all highly susceptible to *A. tumefaciens*. Sunflower proved to be a common host to the two pathogens, but the host responses showed significant differences. The tumors caused by the blackberry organism were smaller and were not accompanied by epinasty of the leaves immediately above the tumor, a characteristic feature associated with tumor formation induced by *A. tumefaciens*. From cultural characters and differences in host range, it was decided that the blackberry organism belongs to *Agrobacterium rubi* (Hildebrand) Starr & Weiss., a species considered as confined to *Rubus* species. Its host range is obviously much wider than this and, as it embraces plants only slightly or not at all susceptible to *A. tumefaciens*, it is likely to prove an important addition to this species in providing material for the study of plant tumors.

Introduction

In planning cytological investigations on plant tumors, it has, first of all, been necessary to find the most suitable material possible. The two most extensive papers dealing with this phase of tumor study, those of Winge (14) and Levine (7), describe observations on the tumors of sugar beet, beet, and tobacco, all of them plants with a comparatively high complement of small chromosomes, little differentiated morphologically. For this and other reasons they furnish unsuitable cytological material. The same objection applies to other species generally used in physiological studies of plant tumors.

Monocotyledonous species, such as those of the genera *Allium*, *Lilium*, and *Tradescantia*, appear to be ruled out, for, as far as I have been able to ascertain, none of them has been found to develop tumors after inoculation with *Agrobacterium tumefaciens*, the standard experimental pathogen. It is true that Jakowska (6) has recently reported infection of onion bulbs with cultures of this organism, but the host response was by no means a typical tumor growth. This author reports the presence of polyploid nuclei in the cells of the inoculated region, but, as will be shown in a subsequent paper, it is quite likely that these represent a normal condition. My own attempts to induce galls with virulent cultures of *A. tumefaciens* failed in the case of species of *Lilium* and *Tradescantia* on which I attempted it.

Of the dicotyledonous species available, one of the most suitable is the broad bean, *Vicia faba*. It has a small complement number ($2n = 12$) of large chromosomes with a sufficient amount of morphological differentiation to

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This investigation was carried out under a Fellowship of the National Cancer Institute of Canada in the Dominion Laboratory of Plant Pathology, Saanichton, B.C.

make cytological abnormalities readily recognizable. The resting nucleus is of the granular type rich in desoxyribose nucleic acid, and the nucleoli are prominent. Although Elliot (4) in her host list for *A. tumefaciens* does not include the genus *Vicia*, she does include three genera of *Leguminosae*, *Pisum*, *Trifolium*, and *Medicago*. It seemed, therefore, highly probable that this species would provide, on inoculation with *A. tumefaciens*, suitable material.

Materials and Results

The cultures of *A. tumefaciens* used by me were kindly supplied by Prof. A. J. Riker of Wisconsin and Prof. D. L. Bailey of Toronto. Their virulence was tested on susceptible species such as tomato, *Nicotiana glutinosa*, and *Datura medeloides*, on all of which there was induced rapidly extensive tumor development. Nevertheless, repeated attempts to induce tumors on stems of young *Vicia faba* plants with these cultures either failed completely, or the growth was so slight as to be hardly distinguishable from an ordinary wound reaction.

Attempts were then made to obtain fresh cultures of *A. tumefaciens* by isolation from tumors found in the field. The most suitable material seemed to be the tumors found on the canes of Himalaya blackberry which were considered locally to be caused by *A. tumefaciens*.

Figs. 1 and 2 show the appearance of natural stem galls or tumors on this host in the field. Fig. 1 shows the more typical appearance on older canes. Fig. 2, which resembles crown gall in type of growth, is more characteristic of tumors on young canes. Fig. 3 shows a large tumor induced on a young cane through needle prick inoculations from a pure culture of the blackberry organism isolated from a tumor such as that illustrated in Fig. 1. The growth here also resembles greatly that induced by *A. tumefaciens* on other hosts.

When the blackberry organism was inoculated through needle pricks into *Vicia faba* stems, there was a rapid development of a tumorlike growth. Within a week, a swelling of the stem in the region of inoculation was apparent, and, within two to three months, extensive tumors developed. Every plant inoculated was affected, and the tumors formed were, usually, very large. Fig. 4 shows a tumor on a stem close to a node, three months and 10 days after inoculation. Here the stem is completely surrounded by the tumor, the inoculation having taken place on the side opposite to that photographed.

A more characteristic growth is illustrated in Figs. 5 and 6, both of which also show views from the stem side opposite to that of the inoculation. Here the inoculations were made at about the mid-internode level. In Fig. 5 the tumor, penetrating into the pith region, has burst the stem on the side opposite to the inoculation, and is already projecting beyond the surface. This photograph was taken about two and one-half months after inoculation. Fig. 6 shows a later stage of a similar tumor taken three months and six days after inoculation. Here the large growth has split the stem so extensively as

PLATE I

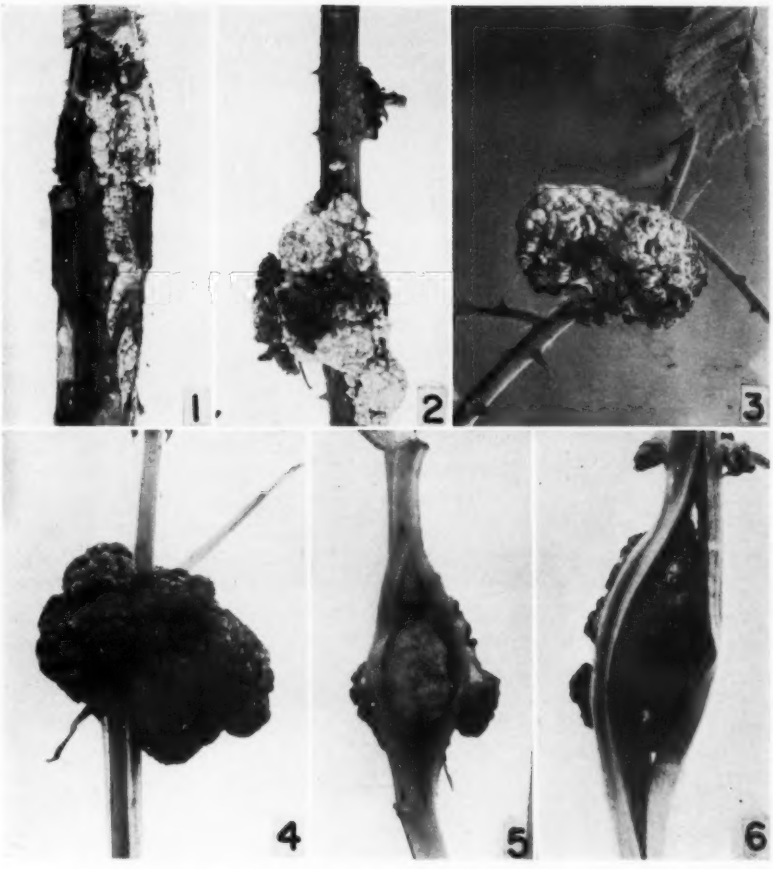

FIG. 1. *Tumors on old cane of Himalaya blackberry in the field.* FIG. 2. *Tumors on young cane of Himalaya blackberry in the field.* FIG. 3. *Tumor on young cane of Himalaya blackberry induced by inoculation from a pure culture isolated from cane tumor.* FIG. 4. *Large tumor on stem of Vicia faba induced by inoculation with pure culture of blackberry organism.* FIGS. 5 AND 6. *Tumors on Vicia faba which have grown through and split the stem.*

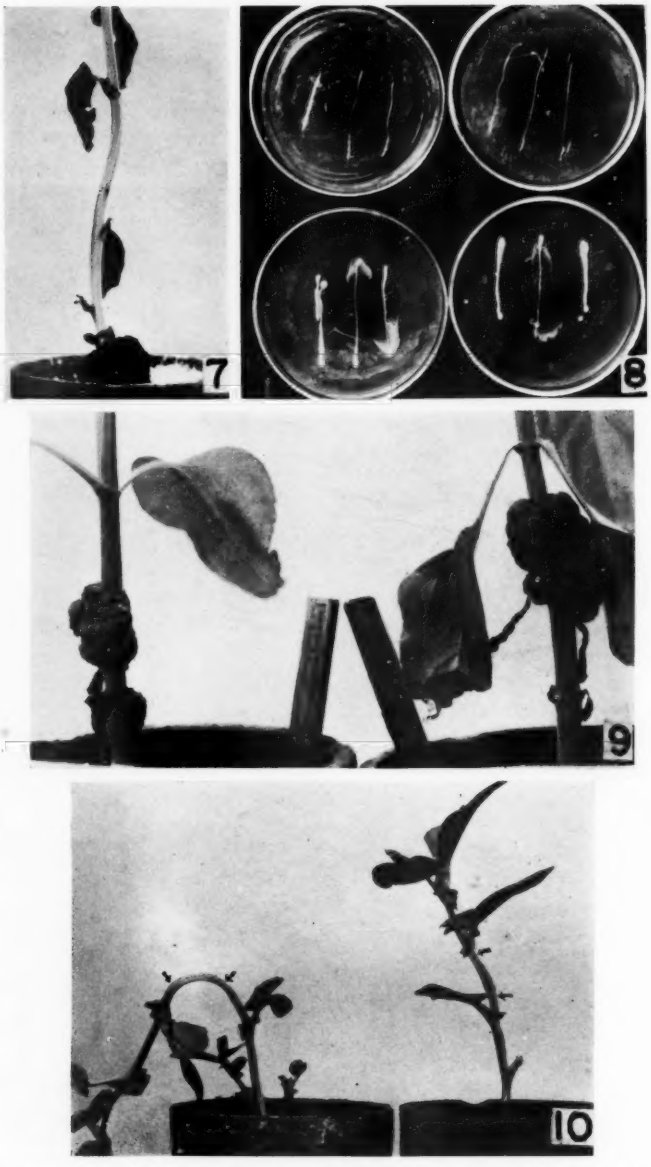

FIG. 7. *Tumor produced at crown of V. faba plant through inoculation with blackberry organism. Note, in this case, the plant has wilted and is dying.* FIG. 8. *Nitrate agar plates inoculated as follows: upper right—blackberry organism re-isolated from V. faba tumor; upper left—blackberry organism re-isolated from sunflower tumor; lower left—A. tumefaciens re-isolated from Nicotiana glutinosa; lower right—subculture of A-6, Wisconsin.* FIG. 9. *Tumors on sunflower plants induced by: left—A. rubi, and, right—A. tumefaciens. Note epinasty on plant to right.* FIG. 10. *Effect of β-indolylacetic acid in lanolin on normal V. faba stem (left) and V. faba stem with incipient tumor induced by A. rubi (right).*

to make light visible above and below the tumor. In still more extreme cases, not photographed, the tumor was attached to the two split stem halves over a distance of less than an inch, leaving large gaps above and below.

The examples so far given are those of stem galls or tumors. However, inoculation at the crown of the plant produced tumors in every way similar, as is shown in Fig. 7. In this and similar cases the effect seemed to be more serious, for, as will be noted, the plant is here wilting. The tumor in this case had turned black and was beginning to decompose, indicating a secondary infection which, I suspect, was the real cause of the plant's death.

My object of obtaining, at will, tumor formation on *Vicia faba* had been accomplished, but I felt I could not leave the matter here. In fact, I had already found a common host for the blackberry organism and *A. tumefaciens* in the sunflower, as is shown in Fig. 9. In this figure the plant to the left was inoculated with the blackberry organism, the one to the right with *A. tumefaciens*. Both were inoculated on the same day and represent fairly typical examples of what was obtained in a whole series of plants. As will be noted, the blackberry organism has induced a distinctly smaller growth in the same time than has *A. tumefaciens*. This paralleled the dissimilarity in the rate of growth on the culture medium used,—potato dextrose agar. A more striking difference in the host plant's reaction is shown in the pair of leaves immediately above the point of inoculation. Those on the plant to the right show the epinasty quite characteristic of the sunflower's reaction to *A. tumefaciens*, which has already been reported by a number of investigators of this organism. The bending of the petioles is not due to a wilting of the leaves, as these remain quite turgid. This epinastic reaction is shown quite soon after inoculation with *A. tumefaciens*, but has never been observed by me as a reaction to the blackberry organism.

Fairly early in the investigation I came to the conclusion that the new organism was distinct from *A. tumefaciens* and, on looking up the literature, found that an organism which, as far as all previous records are concerned, is confined to *Rubus* species, had been described by Hildebrand (5) as *Phytomonas rubi* on the basis of observations made by himself, Banfield (1), and Pinckard (8). This species has been more recently transferred by Starr and Weiss (10) to the new genus *Agrobacterium* erected by Conn (3), the type species being *Agrobacterium tumefaciens*. The species must therefore be considered as *Agrobacterium rubi* (Hildebrand) Starr and Weiss. All these authors give, as one of the distinguishing characteristics of the species, its restriction to *Rubus* species and all attempts made to induce tumors on other hosts have, heretofore, failed. Riker, Spoerl, and Gutsche (9), in their recent comparative study of the gall-forming bacteria, give raspberries and blackberries as the prominent hosts of this organism, but apparently know of no other hosts, as they report none.

Pinckard and Hildebrand list a very considerable number of cultural differences between *A. tumefaciens* and *A. rubi*. I have used but two of these

in an attempt to differentiate between *A. tumefaciens* and the Himalaya blackberry organism. In litmus milk *A. tumefaciens* produces a grayish-brown color, while *A. rubi* gives the typical pink acid reaction. On a nutrient medium in which nitrogen is supplied solely in the form of a nitrate, *A. rubi* does not grow, while *A. tumefaciens* shows a considerable growth. In both cases, the blackberry organism gave a reaction identical with that recorded for *A. rubi*. Fig. 8 shows the difference on nitrate agar. The upper pair of plates were inoculated with pure cultures of the Himalaya blackberry organism, re-isolated from a tumor on *V. faba* and sunflower respectively. No growth has taken place, the white streak on the plate to the left being due to light reflections from the agar cut by the inoculating needle. The two lower plates were inoculated on the same day as the above, respectively from cultures of *A. tumefaciens* re-isolated from *Nicotiana glutinosa* and from a transfer from the original A-6 strain obtained from Professor Riker. Here, the growth along the inoculation streaks is quite considerable. The photograph was taken a week after inoculation. From these results and from further evidence to be adduced, it seems clear the Himalaya blackberry organism is quite distinct from *A. tumefaciens* and belongs to the new species *A. rubi* erected by Hildebrand. If we take into account artificial inoculation, the host range is not at all restricted to *Rubus* species. As stated above, the organism readily produces tumors on *V. faba* and sunflower, two plants widely separated from each other taxonomically. I have also produced tumors on the garden pea, *Pisum sativum*, with ease and regularity, so there seems little doubt that, under favorable conditions, it will attack quite a wide range of host plants.

Although the tumor formation on a common host, sunflower, seems very similar in the case of the two pathogens, there must be, metabolically, a very decided difference between the host parasite relationships in the two cases. The marked differences in growth on culture media would suggest this, as would also the difference in epinastic reaction. The failure of epinasty in the case of sunflower plants inoculated with *A. rubi* suggests the formation of an auxin inactivating enzyme similar to that found by Tang and Bonner (11) in etiolated pea seedlings.

Possibly related to this is a reaction to β-indolylacetic acid shown by *V. faba* stems recently inoculated with *A. rubi*. Based on the reports of Brown and Gardner (2) on the tumor producing effect of applications of β-indolylacetic acid in lanolin to wounded or decapitated stems of various plants, I attempted to hasten tumor growth on *V. faba* after inoculation with *A. rubi* by a similar application. There was no visible effect on tumor formation, but the inoculated stem showed none of the bending characteristic of normal stems treated in the same way. Fig. 10 shows the reaction in the two cases, the arrows indicating roughly the longitudinal extent of the auxin–lanolin smear on the stem. It also shows the extent of the inoculated region in the case of the plant to the right. The inhibition of the bending reaction was not due, as I had suspected, to the development of lignified tissue in the form of tracheids or tracheae, which is

characteristic of plant tumors at a certain stage of development. In this case, the incipient tumors were too young (a week to 10 days old) to show any evidence of tracheid development.

Vicia faba is, unfortunately, not a suitable plant in which to establish the presence of an auxin inactivating enzyme by the colorimetric method used by Tang and Bonner (11, 12), as the presence of an indophenol oxidase in the extracted protoplasm leads almost immediately to a darkening of the test liquid; and there are not the proper facilities in this laboratory for carrying out an *Avena* coleoptile test. It may be that stem tumors induced by *A. rubi* on sunflower, or some other host not yet identified, would prove more suitable for testing the development of auxin inactivating enzymes than those on *V. faba*. In this connection it is interesting to note observations of Thimann (13), who, working with *V. faba* leaves, concluded that, when they were ground with water, the growth substance contained in them was inactivated by oxidizing enzymes. However, this would not explain an inactivation taking place in the plant itself.

The whole question of the relation of specific hormones to plant tumor formation is still an open one* and I believe a comparative study of the physiology of tumors induced by *A. tumefaciens* and *A. rubi* might yield valuable results.

Acknowledgments

Grateful acknowledgment is due to Dr. J. H. Craigie, Dominion Botanist, and Dr. William Newton, Officer-in-Charge, Dominion Laboratory of Plant Pathology, Saanichton, B.C., for providing the facilities for carrying out this investigation.

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* *For the most recent references to this subject see the paper of Armin C. Braun read before the December meeting of the A.A.A.S. and reviewed in Science, 111 : 21. 1950, and that of W. A. Feder abstracted in Phytopathology, 40 : 8. 1950.*

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PHLOEM NECROSIS OF POTATO TUBERS IN RELATION TO LEAF-ROLL-FREE *MYZUS PERSICAE* SULC.[1]

By T. R. Davidson[2]

Abstract

In experiments in which nonviruliferous colonies (25 to 50 individuals per plant) of *Myzus persicae* Sulc. were caged from 7 to 10 days on healthy potato vines growing under field conditions, the tubers failed to develop phloem necrosis. Under comparable conditions, the tubers from healthy plants caged with leaf-roll-infective colonies of this vector developed severe phloem necrosis. When planted, these tubers produced leaf-roll-infected plants.

Potato tuber phloem necrosis has been recognized as one of the symptoms of infection of healthy potato vines by the leaf-roll virus, *Solanum* virus 14. Quanjer (9), in 1913, referred to this condition as "tuber phloem necrosis", and Schultz and Folsom (12), in 1921, called it "net necrosis", as suggested by the brown netted pattern in the flesh of the tuber. The necrosis originates and is most intense in the immediate region of the rhizome scar, and, although it frequently permeates the entire tuber, it tends to be confined to the proximal half.

The incidence of potato tuber phloem necrosis in different varieties susceptible to leaf roll may vary considerably under apparently identical conditions. In certain susceptible varieties, such as Katahdin, this necrosis apparently does not develop under any condition, whereas in others, such as Carter's Early Favourite, it may or may not develop.

According to Elze (5), Murphy and McKay (8), Dykstra and Whitaker (4), Simpson (13), and others, *Myzus persicae* Sulc., *Macrosiphum solanifolii* Ashm., and *Aphis abbreviata* Patch are important vectors of the leaf-roll virus. Apparently all of them agree that *M. persicae* is, in general, a more effective vector than the others. *M. persicae* and *M. solanifolii* have been collected from potato vines in a number of fields at Edmonton (3). The degree of effectiveness of each of these vectors may depend on several factors, including its morphology and feeding habits on the potato vine, the size of the colony involved, and on whether it is a mechanical or a true biological vector.

The physiological relationships associated with the development of tuber phloem necrosis are not yet understood. Quanjer (9) assumed that phloem necrosis in the potato tuber and stem was a direct result of the leaf-roll virus, and he suggested that the necrosis caused starch to accumulate in the leaves. Bawden (1) and others point out that a number of other viruses, which have no visible effect on the phloem, also inhibit the translocation of carbohydrates

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from the leaf. Thung (14) concluded that, as translocation in leaf-roll plants is inhibited before a visible change in the phloem can be detected, the necrosis is a symptom of a change in the phloem brought about by some other cause.

The tomato psyllid, *Paratrioza cockerelli* Sulc., when feeding on vines of potato or tomato, causes (10) very severe vine symptoms, known as psyllid yellows. Also, certain other insects, when feeding on plants, are known to introduce into the plants substances which, although not viruses, cause abnormal conditions or local deformities, such as galls and swellings (7). It was suggested by Sanford and Grimble (11) that one or more of the insect vectors of the leaf-roll virus might, in the absence of the virus, contribute to the incidence of phloem necrosis in potato tubers. These authors refer to certain unpublished co-operative work of Sanford and Manson, who found that *P. cockerelli*, then prevalent in Alberta, was associated with the incidence of a severe type of phloem necrosis of potato tubers that apparently was not of virus origin. Consequently, experiments were undertaken to ascertain whether populations of nonviruliferous *M. persicae*, feeding on potato vines, would cause the incidence of phloem necrosis in potato tubers. A review of the relevant literature did not indicate that this possibility had been determined experimentally.

Materials and Methods

A nonviruliferous population of the green peach aphid, *M. persicae*, was kindly supplied each season by Mrs. J. B. Adams, Dominion Entomological Laboratory, Fredericton, N.B. These insects were multiplied on caged turnip plants. A population of the vector conveying the leaf-roll virus was obtained by raising the insects on caged potato plants infected with leaf roll. Populations consisting of from 25 to 50 insects were transferred, as required, to healthy caged potato plants in the field. The plants were about 6 in. high at the time of the first inoculation. The aphids were allowed to feed on the potato plant for a period of seven days in 1947 and 10 days in 1948. They were then killed by fumigation, the cages removed, and the plants sprayed with a combination of Deenate and Hexaphos. Thereafter application of these insecticides to all plants in the experiment was repeated each week until September, when the danger from aphid infestation had passed.

The date of appearance as well as the intensity of vine symptoms of leaf roll were recorded. At harvest, the tubers from each plant were stored in separate bags in a root cellar, where the temperature range was between 36°–40° F. According to Folsom (6), the optimum storage temperature for the development of leaf-roll tuber phloem necrosis is 45° –50°F. At the end of three months, the tubers were cut and examined for phloem necrosis. The same tubers were again stored, and planted later either in the greenhouse or in the field, and, after about 30 days, checked for the appearance of leaf-roll symptoms in the resulting plants. The greenhouse tests proved to be as reliable as the field tests.

The experiment was repeated on two different dates in 1947 and in 1948, in order to detect any relation between the age of the plant when inoculated with the virus and the incidence of tuber phloem necrosis. There were 10 plants in each treatment. In both years, the sets were planted on May 31. In 1947, the plants were inoculated on July 11 and July 23, and in 1948 on June 28 and July 28.

Experimental Results

The results are presented in Table I. As no vine symptoms of leaf roll developed early on any of the caged or uncaged plants in the control series during the two summers, it appears that the tubers used as seed in these tests

TABLE I

RESULTS FROM CAGING LEAF-ROLL-INFECTIVE AND NONVIRULIFEROUS *Myzus persicae* ON HEALTHY POTATO VINES (10 PLANTS PER TREATMENT)

Date	Treatment	Number of plants positive		
		Vine symptoms	Tuber phloem necrosis	Progeny produced leaf-roll plants
	1947			
July 11	Nonviruliferous aphids*	1	0	2
	Viruliferous aphids*	4	8	10
	Control (caged without aphids)	0	1	1
	Control (not caged)	0	0	3
July 23	Nonviruliferous aphids*	0	1	2
	Viruliferous aphids*	9	10	10
	Control (caged without aphids)	0	0	4
	Control (not caged)	0	0	2
	1948			
June 28	Nonviruliferous aphids**	0	0	0
	Viruliferous aphids**	10	5	10
	Control (caged without aphids)	0	1	1
	Control (not caged)	0	0	0
July 28	Nonviruliferous aphids**	1	2	3
	Viruliferous aphids**	8	10	10
	Control (caged without aphids)	0	1	1
	Control (not caged)	0	0	1

* *and* ** *Plants caged with aphids for 7 and 10 days, respectively.*

were free from the leaf-roll virus. Tests with the tubers borne on these plants, however, revealed that 10 of the plants in 1947 and three in 1948 became infected some time during the growing season. It is probable that the infection resulted from stray viruliferous insect vectors which managed to feed on

these plants, notwithstanding the fact that they had been sprayed. The tubers on only three of these 13 plants developed phloem necrosis. The data also show that the tubers of healthy plants which become infected during the season may or may not develop phloem necrosis. Furthermore, the results indicate that the periodic application of insecticidal sprays to the plants did not give complete protection from infection by viruliferous aphids.

The results obtained from the viruliferous insect series were reasonably definite and conclusive. All of the 40 plants infested with viruliferous aphids became infected with the leaf-roll virus. Furthermore, the tubers on all the plants in the second tests, both in 1947 and in 1948, developed phloem necrosis, whereas, in the first tests in 1947 (July 11) and in 1948 (June 28), the percentage of plants producing necrotic tubers was 80 and 50, respectively.

Of the 40 plants caged with nonviruliferous insects in the four tests, 33 remained healthy. The tubers of three of the seven infected plants developed phloem necrosis. As the percentage of infection in this series was approximately the same as that in the caged and the noncaged controls, it is assumed that the infection was due to accidental infestation by infective aphids after the cages were removed. These data indicate that the nonviruliferous colonies of *M. persicae*, as employed in these experiments, did not cause either the incidence of tuber necrosis or vine symptoms resembling leaf roll.

Whether or not, in the process of feeding, aphids, nonviruliferous or otherwise, introduce chemical substances into the plants that act with the virus to induce phloem necrosis in the potato tuber, could not be determined from the results obtained. At any rate, it may be assumed that such chemical is not autocatalytic, and that the amount of it injected is largely dependent on the size of the colony on the plant. There appears to be no experimental evidence on this point reported in the literature.

Even if there is no biological interdependence of the leaf-roll virus and the vector, certain factors of the environment may possibly influence the infection of the host by the leaf-roll virus and the development of phloem necrosis in the tubers. For example, strong sunlight (11) favored the rapid development of phloem necrosis in tubers borne on potato vines populated with *P. cockerelli*. Also, the pretreatment of either bean or tobacco plants for a time in subdued light rendered them more susceptible to the tobacco necrosis virus (2).

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STUDIES ON THE PARASITISM AND VARIATION OF *ALTERNARIA RAPHANI*[1]

By R. G. Atkinson[2]

Abstract

The natural infection of radish seed with *A. Raphani* may result in a lack of germination, a pre- or postemergence blight, a distinctive lesioning of cotyledons and hypocotyls, the presence of scablike lesions on table radish, and in the spotting and blighting of leaves, stalks, and siliques. The fungus was isolated from the internal tissues of all parts of dormant radish seed. Although the pathogen has been reported only on radish in Canada and the United States, the present investigation shows that Canadian isolates are capable of causing a severe leaf blight of stocks and wallflowers. Under field conditions at St. Catharines, Ont., most rapid progress of the disease occurred at temperatures within the optimum range for the fungus, i.e., 22° to 26° C. Experimental evidence suggests that *A. Raphani* does not establish an overwintering inoculum in the soil by means of diseased plant debris. Increased soil moisture was associated with increased seedling disease. At a high soil moisture content, infection was lowest at 18° C.; at medium soil moisture, it was lowest at 18° C. and also at 23° C., the next highest experimental temperature.

Monosporous isolations showed the presence of numerous wild type strains of *A. Raphani* which were closely related culturally. Five of these studied intensively differed widely in virulence and sporulation, but had similar growth rates and nutritional requirements for maximum growth. Although most isolates of *A. Raphani* produced only a few spores in ordinary agar cultures, abundant sporulation was obtained by wounding plate cultures and removing the lids of the culture plates. In agar culture, the wild types readily produced mostly appressed variant strains also showing close cultural relations. These variants exhibited wide differences in pathogenicity, rate of growth, and nutritional requirements, but all showed practically complete loss of sporulation either in normal or wounded cultures. The effects of cultural variation of wild type strains on cultural habit, pathogenicity, rate of growth, sporulating capacity, and nutritional requirements were random and unrelated. These data, as well as the spontaneous origin and irreversibility of the variants, favored the view that they arose in culture by mutation in the naturally occurring strains.

A. Raphani was shown to be capable of surviving at least 18 months in dry soil cultures with no loss of cultural habit, virulence, or sporulation.

Appreciable increase in emergence and decrease in seedling infection was obtained by seed treatments with some of the common fungicidal dusts.

Introduction

During the course of mycological analyses of seed submitted for Elite, Registered, and Certified status, a number of *Alternaria* species have been isolated, principally from seed of Cruciferae and carrot. Of these parasites, *Alternaria Raphani* has been isolated from radish (*Raphanus sativus* L.) and named a new species by Groves and Skolko (3). They have pointed out the wide geographic range of the fungus which in Canada and the United States has been isolated only from radish. McLean (6) considers that infection by

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A. Raphani is the most important factor in low germination of radish seed in Michigan. Neergaard (8), in Denmark, recognized the fungus as a new species, and, owing to wartime conditions, unaware of Groves and Skolko's work, named it *Alternaria Matthiolae*. He acknowledges, however, the priority of *A. Raphani* as the proper name of the organism.

The presence of the pathogen in radish seed from all parts of Canada and reports of its damage to seed crops indicated the importance of this species and prompted the present investigation of the fungus. This paper describes the symptoms on radish resulting from natural seed-borne infection with the fungus and on stocks and wallflowers resulting from artificial inoculations. The effect of environmental factors on the parasitism of the fungus on radish was also investigated. The variation of *A. Raphani*, both in nature and in culture, was studied intensively together with its effects on pathogenicity, temperature relations, sporulation, and nutritional requirements. Finally, results of seed treatment tests for the control of *A. Raphani* are reported.

Host Range and Symptoms

A. Host Range

In Canada (3) and the United States (6) *A. Raphani* has been found only on radish. In England, however, Ware (11) reported what undoubtedly was the same *Alternaria* as causing a leaf spot of stocks under greenhouse culture. Neergaard (8), in Denmark, isolated the fungus from plants and seeds of stocks and radish, and from seeds of cabbage (*Brassica oleracea* var. *capitata* L.). By artificial inoculations, he showed that the pathogen could also attack wallflower (*Cheiranthus Cheiri* L.) and *Iberis amara* L. In addition, one of his isolates attacked lettuce seedlings (*Lactuca sativa* L.). Groves and Skolko (3) successfully inoculated seedlings of radish, cauliflower (*Brassica oleracea* L. var. *botrytis* L.), swedes (*B. napobrassica* Milb.), and cabbage. They failed to obtain infection on wallflower. It seemed advisable, therefore, to determine the pathogenicity of Canadian isolates on stocks and also to test again their action on wallflowers.

B. Symptoms on Radish

1. *Seedlings*

A distinctive seedling symptomatology resulted when naturally infected radish seed was sown in the greenhouse, either in sterilized or unsterilized soil. Soil-borne fungi did not interfere with seedling disease expression. In their study of germination and seedling diseases of cereals, Machacek and Wallace (5) had a similar experience and concluded also that nonsterile soil, if properly handled, was as satisfactory as sterile soil.

The use of heavily infected seed gave rise either to a pre-emergence or post-emergence blight, as shown in Pl. I, Fig. 1. The presence of lesions on the cotyledons and upper hypocotyl distinguishes this postemergence blight from the more general damping-off diseases of seedlings induced by soil fungi.

A. Raphani was isolated consistently from blighted seedlings as well as from many ungerminated seeds.

Unless severely infected, most of the seedlings survived. Before the appearance of the primary leaves, grayish-green or brownish-olive to black lesions were observed on the cotyledons, usually at the median indentation of the margin, as illustrated in Pl. I, Fig. 2. When large areas of the cotyledons were infected, the seedlings usually died. Less frequently, small black dot-lesions appeared on the cotyledons, as shown in Pl. I, Fig. 4. Black stripe- or fleck-lesions occurred throughout the length of the hypocotyl or were restricted to the basal transition swelling. Coalescence of the lesions in this part resulted in a black collar around the base of the hypocotyl. Pl. I, Fig. 3 shows various degrees of hypocotyl lesioning.

2. *Table Radish*

Seedlings with severely infected hypocotyls usually formed table radish with black, scablike lesions on opposite sides of the upper half of the "root", as shown in Pl. I, Fig. 5. No rot developed, as the fungus invaded only the outer rings of the xylem tissue.

3. *Mature Plants*

The following symptoms are based on observations of naturally infected plants in the field. McLean (6) has described symptoms based on inoculations.

Lesions occurred on all leaves; the younger smaller spots were usually black, whereas the older ones were "buffy-olive"* to "brownish-olive" and were dry and papery, with a slightly raised marginal ridge. They varied from one millimeter or less to slightly more than a centimeter. Individual lesions, especially at the margins, frequently coalesced to form large necrotic areas which on drying tended to tear. Typical leaf lesions are shown in Pl. I, Fig.6. On the petioles, lesions appeared as numerous or few black dots and flecks, chiefly on the underside, as seen in Pl. II, Fig. 2. Large petiole lesions were roughly elliptical and sunken, and measured 1 to 6 cm. long and 1 to 1.5 cm. wide.

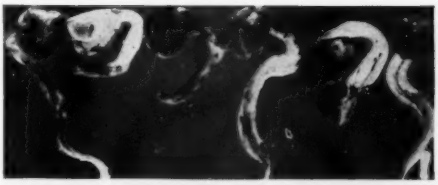
Lesions on the siliques were at first small black dots with a water soaked halo. They developed into larger circular lesions with netlike margins and occasionally with yellowish halos, as shown in Pl. II, Fig. 7. In severe infections the whole silique was blighted, resulting in small shrivelled seed.

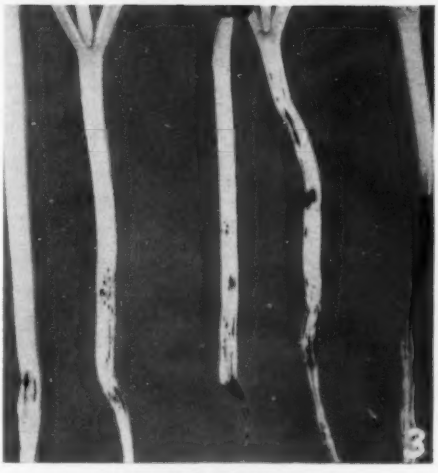
On the seed stalks small lesions appeared as black, dot- or flecklike spots, whereas larger ones were elliptical and sunken, as depicted in Pl. II, Fig. 6. Upper seed stalks of small diameter were frequently killed.

* *Ridgway. Color standards and color nomenclature. Washington. 1912.*

Natural seed-borne infection on radish due to A. Raphani. FIG. 1. *Pre-emergence blight.* FIG. 2. *Marginal lesions on cotyledons.* FIG. 3. *Various degrees of lesioning on hypocotyls.* FIG. 4. *Dot type of lesions on cotyledons.* FIG. 5. *Scab lesions on table radish.* FIG. 6. *Lesions on leaf.*

PLATE I





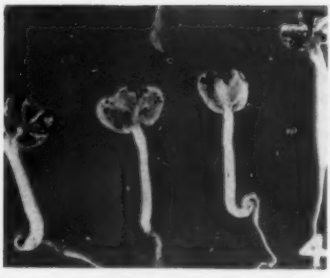
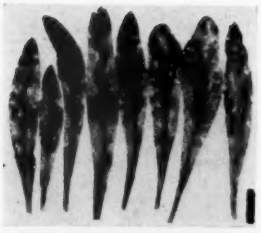
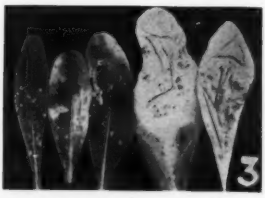


PLATE II





4. *Seed Infection*

Table I presents the incidence of the pathogen in dissected parts of diseased seed of radish var. Scarlet Globe when plated on agar. The cotyledons were halved along the fold line so that four pieces were plated from each seed,

TABLE I

ISOLATION OF *A. Raphani* FROM THE INTERNAL PARTS OF RADISH SEED

Method	No. seeds dissected		Internal parts	No. pieces			
				Plated		Infected	
	(1)	(2)		(1)	(2)	(1)	(2)
Seed disinfected before dissection	18	20	Cotyledons	72	80	11	9
			Radicle *plus* plumule	18	20	3	4
			Testa	36	20	18	3
Parts disinfected after dissection	20		Cotyledons	80		4	
			Radicle *plus* plumule	20		2	
			Testa	20		1	

whereas each testa was either halved or plated intact. The fact that the pathogen grew from parts surface disinfected, as well as from parts untreated, after dissection, indicates that *A. Raphani* is capable of colonizing the internal tissues of all parts of radish seed. This explains why, in heavily infected samples, many seeds fail to germinate or succumb to pre- or postemergence blight.

Table II shows that the presence of *A. Raphani* in radish seeds is associated with serious reductions in emergence. The same infected sample was used both in the greenhouse and field tests and, just prior to sowing, gave 52% *A. Raphani* in an agar plate test. The healthy seed samples contained no pathogenic fungi. Similar evidence is presented in the untreated series of seed treatment tests (Tables VIII and IX).

C. Symptoms on Stocks and Wallflowers

Nine-week-old Brompton (*M. incana* R. Br. var. *annua* Voss) and Ten Weeks (*M. incana* R. Br. var. *autumnalis* Voss) stocks and 10-week-old Finest Double Mixed wallflowers were inoculated with *A. Raphani* by atomizing with spore suspensions of monohyphal-tip isolates.

Infection on radish, stocks, and wallflowers due to A. Raphani. FIG. 1. *Lesion on leaves of Double Mixed wallflowers artificially inoculated.* FIG. 2. *Lesions on petioles of lower leaves (lamina removed) of radish due to natural seed-borne infection.* FIG. 3. *Lesions on leaves of Ten Weeks stocks artificially inoculated.* FIG. 4. *Ten Weeks stocks artificially inoculated.* FIG. 5. *Double Mixed wallflowers artificially inoculated.* FIG. 6. *Lesions on seed stalks of radish due to natural seed-borne infection.* FIG. 7. *Lesions on siliques of radish due to natural seed-borne infection.*

TABLE II

THE RELATION OF POOR EMERGENCE OF RADISH SEED TO INFECTION WITH *A. Raphani*

Infected seed				Healthy seed	
Sterilized compost in greenhouse		Sandy loam in field		Sandy loam in field	
Flat No.	Emergence, %	Row No.	Emergence %	Sample No.	Emergence, %
1	45	1	35	I–619	82
2	32	2	39	I–640	78
3	47	3	51	I–618	82
		4	44	I–617	69
		5	29	I–649	84
Av.	41	Av.	40	Av.	79

The inoculated stocks were incubated for two days at a relative humidity of from 75 to 85% and a temperature of from 62° to 68° F. in Wisconsin tanks covered with large window panes and lined with wet sacks. The plants were then removed to a controlled air temperature of from 66° to 68° F.

Because of high summer temperatures, the inoculated wallflowers were placed under bell jars in the cellar, and in adequate window light, for two days at from 68° to 72° F. After removal of the bell jars the plants were left under these conditions.

1. *Stocks*

Three days after inoculation pale gray to grayish-green, sunken dot-lesions measuring from 0.5 to 1.5 mm. appeared on the leaves. Coalescence of these spots formed circular to rectangular areas approximately 10 mm. long and from 2 to 5 mm. wide. In time the apical portions of the lower leaves drooped, appearing at first pale green and flaccid, and after 12 days became yellow, dried, and shrivelled. The lower and middle leaves were the most susceptible, the lower ones eventually abscissing or becoming easily detached. On green leaves yellowish-green halos surrounded the dot-lesions. Both varieties were equally attacked. Symptoms are shown in Pl. II, Figs. 3 and 4.

2. *Wallflowers*

Within 36 hr. after inoculation, incipient pale grayish-brown dot-lesions, about 0.25 mm. in diameter and frequently surrounded by yellowish-green halos, appeared on all plants. After 60 hr., the lowermost leaves and the apical portions of the middle ones were flaccid, grayish-green, and drooped. In addition, withered grayish-green areas occurred in scattered patches, often at the margins, which then became puckered. The presence of the yellowish green halos around the numerous dot-lesions resulted in a mottling effect. After six days, although the more heavily attacked leaves were yellow and blighted, most were green or only slightly yellow. Lesions occurred on the

midveins and were larger on the proximal half, appearing as elongate-elliptical areas, 27 mm. long and from 1 to 2 mm. wide. After 16 days, four plants were practically dead, the remaining eight severely blighted. Symptoms are illustrated in Pl. II, Figs. 1 and 5.

Effect of Environment on Disease Incidence in Radish

A. Soil Moisture and Temperature

To determine the effect of these factors on disease incidence in seedlings from naturally infected seed, Wisconsin tanks were adjusted to operate at 10°, 14°, 18°, 23°, and 28° C., and natural sandy-loam soil was watered to give relatively low, medium, and high moisture contents. At each temperature, two cans of soil at low, four at medium, and two at high moisture content were each sown with 100 radish seeds var. Scarlet Globe naturally infected with *A. Raphani*. Three experiments were conducted, the first being merely a preliminary one and not reported here. Since it was felt that the effects of soil temperature and moisture might vary according to degree of infection, a moderately and a severely infected seed sample was sown in Experiments II and III, respectively.

In Experiment II, the three soil moistures were 24, 32, and 39%, and, in Experiment III, 21, 26, and 37% of the moisture-holding capacity. In order to maintain the experimental temperatures and moistures of the soil around the germinating seeds more accurately, discs of waxpaper were laid over the surface, and sheets of waxpaper and newspaper covered the tanks until emergence. The thesis that soil temperature and moisture might be most decisive during the pre-emergence period was supported by the isolation of the pathogen from all internal parts of radish seed. The soil moisture levels were maintained by adding water to a deep, central well until the original weight was restored.

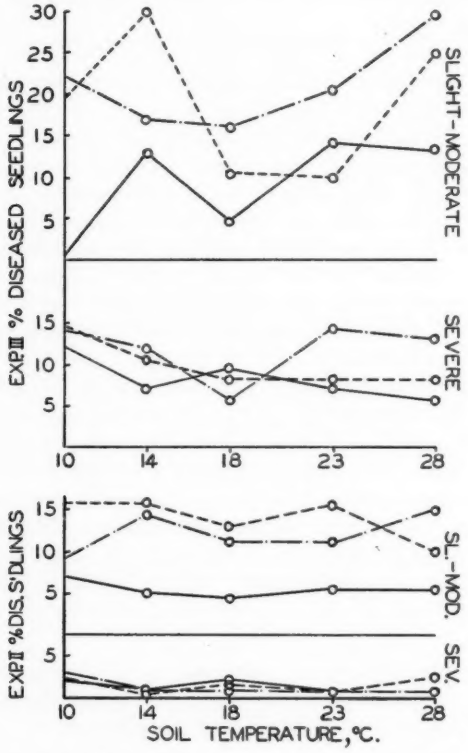
The seedlings were pulled and examined at 10 days as the preliminary experiment showed that after this period invasion by soil organisms, especially at the higher temperatures, might interfere with seed-borne lesioning.

The same soil was used in the three experiments as when healthy seed was sown in soil of the preliminary experiment, the resultant seedlings were free from disease. Evidently the sowing of diseased seed did not result in the establishment of *A. Raphani* in the soil.

Results of Experiments II and III are shown in Text-fig. 1. Evidently soil temperature had no appreciable effect on disease incidence in seedlings from only moderately infected seed. Soil moisture, on the other hand, was limiting, as a much smaller number of seedlings were lesioned at the low than at the two higher soil moistures.

When more heavily infected seed was sown, total disease counts were again lowest at the low soil moisture, with soil temperature having relatively little effect. At the higher moisture levels, however, soil temperature did have an

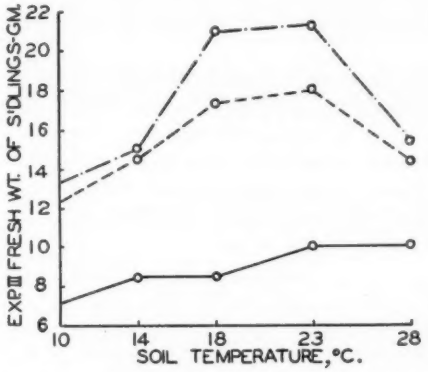
effect: at high soil moisture, least disease occurred at 18° C.; at medium soil moisture, the disease minimum occurred at 18° C., and also at 23° C.—the next highest experimental temperature. At higher and lower temperatures, the percentage of lesioned seedlings increased sharply, being greatest, with one exception, at high soil moisture.



TEXT-FIG. 1. *The effect of soil temperature and moisture on seedling infection in radish grown from seed naturally infected with a relatively moderate (Expt. II) and a high (Expt. III) incidence of A. Raphani.* O——O *Low soil moisture.* O- - -O *Medium soil moisture.* O—·—O *High soil moisture.*

Text-fig. 1 shows the analysis of these effects on total disease counts in terms of degree of infection. In the high soil moisture series, soil temperature affected both the "slight-moderate" and the "severe" infection categories of diseased seedlings. At medium soil moisture, however, only the "slight-moderate" infection category was affected. Although soil temperature had only a relatively slight effect on total disease counts at low soil moisture, a sharp reduction in the incidence of the "slight-moderate" infection category occurred at 18° and 10° C.

Text-fig. 2 records the fresh weights of the seedlings in Experiment III. They show an inverse correlation with disease incidence, except at high soil moisture, where infection increased sharply at 23° C. although seedling weight remained the same as at 18° C.



TEXT-FIG. 2. *The effect of soil moisture and temperature on the fresh weight of seedlings grown from seed heavily infected with A. Raphani.* O———O *Low soil moisture.* O- - - -O *Medium soil moisture.* O—·—O *High soil moisture.*

Although healthy seeds were not sown to determine the optimum conditions for host development, it was possible to observe that such conditions occurred at 18° C. and 23° C., both at medium and high soil moistures respectively.

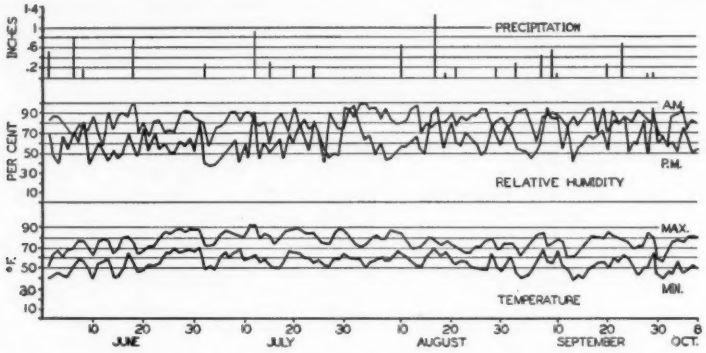
Soil temperature and moisture had little effect on emergence except for a marked reduction at 10° C. at low soil moisture, where at other temperatures only a slight decrease was evident.

B. Meteorological Conditions

To determine the effect of meteorological conditions, a plot of naturally infected radish was under continuous observation from the emergence of seedlings to the maturation of the siliques. Naturally infected radish seed var. Scarlet Globe was sown on June 24. Meteorological data are presented in Text-fig. 3.

By July 11, spores of *A. Raphani* were found on lesions on the cotyledons. No leaf infection was evident. Very high temperatures had prevailed with only a trace of rain. On July 30, lesions were observed on many of the lower leaves. As the meteorological graph indicates, rain occurred four times between July 11 and July 30. The logical inference is that spores had been splashed from the cotyledon lesions to the lower leaves. Not until Aug. 19, when many plants were in bloom and a few siliques had developed, did lesions appear on the middle leaves. At this time, extensive lesioning was present on the lowest leaves, but no lesions were evident on the flowers or siliques.

By Sept. 4, lesions were present on the upper small leaves associated with the siliques. On a few plants bent to the ground, small lesions were observed on siliques close to lower diseased leaves. Most siliques, however, remained



TEXT-FIG. 3. *Meteorological data during progress of the disease in field plots of radish grown at St. Catharines, Ont., from seed naturally infected with A. Raphani.*

healthy. Between Aug. 19 and Sept. 4, the temperature was consistently lower than during the previous period. This cooler weather, together with four periods of rainfall, evidently favored extensive infection of the upper leaves. During the remainder of September, a few lesions were observed on the upper seed stalks.

By Oct. 2, extensive lesioning of the siliques was evident, but it was most severe on those bent toward the lower leaves. Extensive infection was also present on the small leaves of stalks bearing siliques. Apparently the lower temperatures and moderate rainfall in September had favored the final stages of the disease and resulted in the infection of the siliques and the seeds they contained. The siliques were still green when infected, a fact ruling out the possibility that they were attacked only after senescence and ripening had been initiated.

These observations show, as might be expected, that the secondary cycle of infection was initiated by means of spores blown or splashed from the primary lesions on the cotyledons to the lower leaves. The disease remained confined to these parts until late summer temperatures dropped mostly to about 80° F. or somewhat lower, the pathogen then spreading rapidly to the upper leaves and the nearby siliques.

It remained to determine whether or not the infested residue from such a diseased crop would establish an overwintering soil-borne inoculum capable of attacking healthy seedlings in the following season.

C. Overwintered Host Debris in the Soil

Infected leaves and stems from this diseased radish crop were incorporated with sandy loam soil from around the plants. Seven flats of this prepared soil

together with a flat of similar control soil without the diseased material were overwintered outside from October until the end of May of the following spring. At that time, each flat was sown with 200 healthy appearing radish seeds. In addition, a second flat of control soil gathered some distance from the previous site of the diseased crop was similarly sown. After from 18 to 20 days, the seedlings that developed were removed and examined for the presence of lesioning.

In all flats of soil incorporated with diseased material, most of the seedlings were stunted, with small pale cotyledons, and showed light brown to blackish brown lesions on the hypocotyl at or below ground level. Many were completely girdled at this point by a soft pale-brown cortical rot and usually were collapsed on the surface of the soil. Many stunted seedlings were abnormally twisted. Numerous isolations from lesioned seedlings consistently gave *Rhizoctonia* sp., *Fusarium* spp., and an unidentified "sclerotial fungus", which produced only numerous small sclerotia.

Seedlings in the flat of uninfested control soil from the radish plot, although somewhat more vigorous than those in the infested soil, also showed basal hypocotyl lesions from which *Rhizoctonia* sp., *Fusarium* spp., and two colonies of *A. Raphani* were isolated. The isolation of the pathogen was considered to be due to the chance inclusion of infected seed and not to soil-borne inoculum. This view was supported in later experiments by the isolation of *A. Raphani* from seedlings in control soil not associated with the radish crop.

Seedlings in this control soil showed the healthiest, most vigorous development, but even a few of them showed small lesions at the base of the hypocotyls which yielded *Fusarium* spp. and the "sclerotial fungus."

Similar results were obtained when the same soil was sown with the same seed lot in two consecutive plantings immediately after the first was harvested.

Evidently lesioning of the seedlings was due to several soil fungi. These, along with other microorganisms, through their competitive or antagonistic actions, rendered the introduced pathogen innocuous as a potential soil parasite. These experiments strongly suggest that, in this locality at least, *A. Raphani* does not establish inoculum in the soil by means of overwintered host debris from diseased radish crops.

Variation of *A. Raphani*

Although many isolations of *A. Raphani* from infected seed and diseased plants were made, the original colonies that developed always had well developed aerial mycelium of varying colors, such as "olive-gray", "iron-gray", or "greenish-olive". When transferred to malt agar plates after from three to 10 months on potato-dextrose-agar slant cultures, most of the colonies developed sectors of appressed mycelium and irregular areas of white aerial growth, as illustrated in Pl. III, Fig. 1. This result suggested that *A. Raphani* is unstable in agar culture and that the areas of aberrant mycelium were composed of variant strains derived from the initial isolates from nature. It was

decided, therefore, to determine whether or not various strains of *A. Raphani* existed in nature and to study the pathogenic, physiologic, and nutritional properties of these and any variants of them that might arise during culture.

A. Naturally Occurring Strains of *A. Raphani*

Single spore isolations of *A. Raphani* were made from mature radish plants grown in the field from naturally infected seed. Diseased leaves were usually held in a moist chamber overnight to secure abundant sporulation. Loopfuls of a spore suspension, made in sterile water on a flamed slide, were allowed to make parallel streams down an agar plate held vertically. After overnight incubation at room temperature, suitably isolated single germinating spores were cut out and removed to potato-dextrose-agar plates, 12 per plate, and placed at 26° C. In this manner a total of 312 monosporous isolates were obtained.

The marked differences in the single spore colonies that developed, often in the same Petri dish, and their consistent appearance during the isolations, indicated the existence in nature of cultural strains of the pathogen. Based on these initial characteristics, the 312 isolates were grouped into 13 strains.

Two strains predominated, one comprising 129 isolates or 41.3%, the other comprising 89 isolates or 28.5%, of the total number studied. The isolates could be classified roughly into three main groups: the first forming pulvinate colonies of smooth or only slightly rough profile and abundant aerial mycelium to the edge; the second producing colonies with a tufted, central column rising above a rather flat layer of aerial mycelium bordered by a relatively narrow appressed, greasy-appearing margin; and the third developing colonies similar to the second but lacking the tufted column. Other variations within these groups consisted of color differences in the upper and under surface of the colonies and the presence or absence of zonation and speckling due to the formation of chlamydospores.

After 14 to 19 days, most of the colonies either failed to produce spores or formed only a relatively few small ones with short beaks. Some strains produced short chains of smooth, light to dark smoky-olive chlamydospores, whereas others formed either short chains or from two- to five-celled aggregates of thick-walled, dark brown to brownish-black, verrucose to tuberculate chlamydospores. It should be remembered that the foregoing characteristics represented the initial cultural expression of the naturally occurring strains in their first artificial environment. Henceforth, these naturally occurring strains will be referred to as the 'wild types', this term being used in the same sense as Miller (7) used it in his studies on cultural variation of the *Fusarium* of muskmelon wilt. As preliminary experiments showed that wild type isolates a few months old had produced new or variant mycelia, it was felt advisable to isolate such cultural variants and to make a comparative study of these and the original naturally occurring types.

B. Cultural Variants from Wild Type Strains

From each colony of the original monosporous isolates from nature, mass transfers had been made, at from 12 to 21 days, both to slants of potato dextrose agar and to tubes of moistened sterile loam soil. The latter was included to compare the relative stability of the pathogen in the two vastly different environments. After from 13 to 19 weeks, the various strains were transferred from the agar slant and soil tube co-cultures to potato-dextrose-agar plates. Most of the colonies that developed showed patches of white aerial growth or sectors of appressed mycelium. Evidently these represented cultural derivatives from the wild type as they had never appeared in the initial isolates from nature.

The wild type and its cultural variant in each colony were separated by a technique ensuring their purity upon initial isolation. As few or no spores were produced, small portions of hyphae of the desired strain were picked off the heterogeneous colonies into sterile water on a flamed slide. The hyphae were macerated by cutting with a microscalpel made from ordinary nichrome wire. Loopfuls of the resulting suspension of hyphal fragments were then transferred to agar plates held vertically and allowed to stream down them as previously described for spore suspensions. After overnight incubation at 26° C., single tips of germinating hyphal fragments were severed with the microscalpel and transferred to plates of potato dextrose agar, six of the wild type and six of its cultural variant per plate. The colonies that developed in each plate were of two distinctly different kinds, the original wild type and an entirely new strain never encountered in original isolations from nature.

1. Variants from Agar Culture

When subcultured on potato-dextrose-agar slants for 13 to 19 weeks, 15 of the 18 wild type isolates studied produced cultural variants. The monohyphal-tip isolates of these were mostly appressed and whitish to creamish, in striking contrast to the aerial pigmented colonies of the wild types. Pl. III, Figs. 4 to 8 shows five wild type strains and their respective variants after transfer, either directly from the initial monohyphal-tip colonies, or from young subcultures of these, to potato-dextrose-agar plates. Such mass transfers resulted in the production of considerable pigment in the variant from wild type IVS4A,* only a slight amount in the variant from IS2 and IIS6, and none in the variants from IS1 and IIIS4C. The original monohyphal-tip isolates of the variants also frequently developed slight olive pigmentation with age.

2. *Variants from Soil Cultures*

Compared to their behavior in the agar slant subcultures the wild type isolates studied were much more stable in the duplicate set of soil tube cultures, since only six of a total of 15 gave rise to cultural variants. They were,

** In the notation of the wild type strains, the Roman numerals refer to a certain series of isolations; the letter "S" and arabic numerals to distinctly different monosporous strains. When present, the final letter indicates some degree of cultural affinity of the strain to one similarly numbered.*

however, essentially like those derived from the agar slant cultures. One of the variants derived from a soil culture together with its respective wild type is shown in Pl. III, Fig. 3.

3. *Stability of the Cultural Variants*

To determine the stability of the cultural variants, the initial monohyphal-tip isolates of five of them derived from the agar cultures were transferred to potato-dextrose-agar slants. After from 10 to 13 weeks, 24 single hyphal tips were isolated, in the manner previously described, from the slant cultures of each variant. The fact that the resultant colonies were practically identical with the original monohyphal-tip isolates of the variants indicated that they had remained unchanged during the experimental period. On the other hand, as they had arisen spontaneously in wild type cultures during a similar interval, they evidently represent stable discontinuous variations such as characterize mutations. A comparative study of certain wild types and their cultural variants was undertaken and concerned their pathogenicity, sporulation, temperature relations, prolonged survival in dry soil cultures, and nutritional requirements. Five wild type strains were selected for study and these, with one exception, were the ones that were most frequently isolated and that exhibited the greatest cultural differences among the 13 strains identified.

C. Pathogenicity of the Wild Type Strains and Their Cultural Variants

The pathogenicity of the wild type strains and their cultural variants was determined by inoculating potted plants of stocks and also sterile seedlings of radish, stock, and wallflower.

1. *Potted Plant Inoculations*

One pot of seven-week-old Ten Weeks, and one of Brompton, stocks were inoculated with each of the wild type and variant strains by placing portions of agar cultures on the middle, lower, and rosette leaves. The inoculum was from young subcultures of the original monohyphal tip isolates of the wild type and variant strains. After seven days in the Wisconsin tank humidity chambers previously described, the plants were removed and the results recorded on the eighth and seventeenth days. Table III gives the disease rating assigned to each strain.

Three wild types, IS2, IVS4A, and IIS6 were extremely virulent, all causing severe withering and the eventual death of the inoculated leaves. In marked contrast, the wild type IS1 was only weakly pathogenic, producing only a few small lesions. The fifth, IIIS4C, although causing some withering of a few lower leaves, induced an intermediate moderate infection on the others, which remained green and turgid.

The cultural variants of the virulent wild types IS2 and IIS6 showed a complete absence or only a trace of parasitism. On the other hand, the variant from the third virulent strain IVS4A was as parasitic as the wild

TABLE III

DISEASE RATINGS OF FIVE WILD TYPE STRAINS OF *A. Raphani* AND THEIR CULTURAL VARIANTS, BASED ON INOCULATIONS OF POTTED STOCK

Strain	Disease ratings			
	Ten Weeks var.		Brompton var.	
	Wild type	Variant	Wild type	Variant
IS1	+*	++	+	++
IIS2	++++	+	++++	No infection
IVS4A	++++	++++	++++	++++
IIIS4C	+++	++	+++	++
IIS6	++++	+	++++	+

* + = *trace of infection;* ++ = *slight infection, larger lesions;* +++ = *moderate infection, some leaves withered;* ++++ = *severe infection, all inoculated leaves withered or dead.*

type, causing complete killing of the lower inoculated leaves. The variant from the moderately pathogenic strain IIIS4C was only slightly less parasitic than the wild type. Finally, and of most interest, was the fact that, although the strain IS1 produced only a few incipient lesions, its cultural variant showed an appreciable increase in parasitism.

To determine in a quantitative manner these differences in pathogenicity, seedlings of radish, stocks, and wallflowers were inoculated under aseptic conditions in test tubes.

2. *Test Tube Seedling Inoculations*

Small filter-paper cylinders, with the uppermost end closed, were pushed into large test tubes (17 mm. wide), approximately one-third full of distilled water, until they projected only slightly above the surface of the water. The tubes were then plugged and autoclaved. Uniform and healthy appearing seed of radish (var. Scarlet Globe), stock (Ten Weeks and Brompton), wallflower (Finest Double Mixed), and lettuce (New York Special No. 12) was surface sterilized, rinsed in sterile distilled water, and placed aseptically on the ends of the cylinders in the prepared tubes. Lettuce was included as Neergaard (8) reported one of his isolates attacked this host. Five seeds of radish and of stock, 10 of wallflower, and seven of lettuce were sown in each respective tube and allowed to germinate and grow at room temperature close to a window. The radish seedlings were inoculated after five, the Ten Weeks stock after eight, the Brompton stock after 14, and the wallflower and lettuce after 10 days with bits of agar cultures. Five tubes of radish, wallflower, and lettuce, three of Ten Weeks and two of Brompton stocks were inoculated with each of the five wild type strains and their cultural variants. The inoculum was from monohyphal-tip colonies or from young subcultures of these. Tubes of control seedlings were inoculated with pieces of sterile agar. The inoculated seedlings were left at room temperature in front of a

window. Results were recorded from 14 to 16 days after inoculation and are presented in Table IV and Text-fig. 4. The control seedlings showed no sign of deterioration resulting from their confinement in the tubes.

TABLE IV

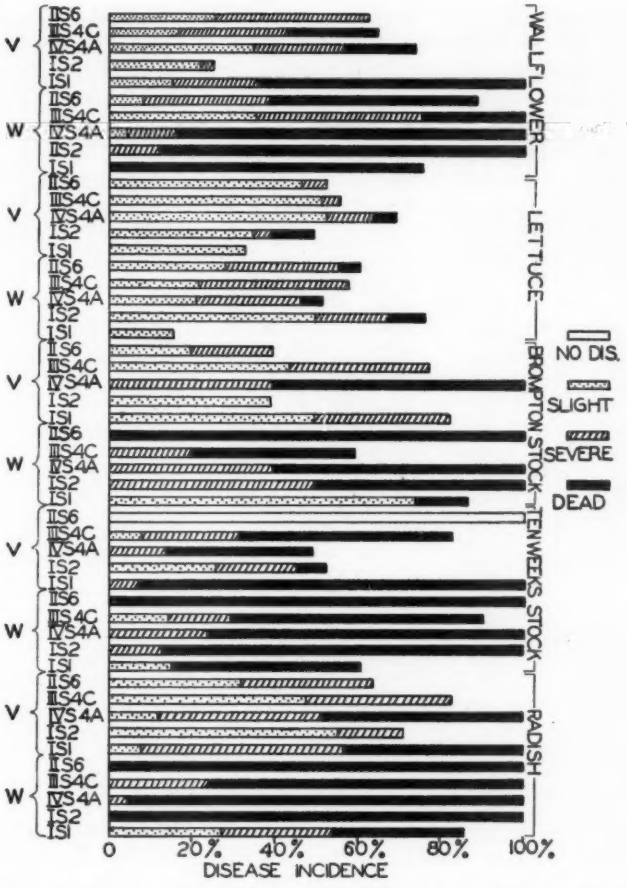
RESULTS OF INOCULATING SEEDLINGS WITH WILD TYPE STRAINS OF *A. Raphani* AND THEIR CULTURAL VARIANTS UNDER STERILE TEST TUBE CONDITIONS

Host	Strain	Culture age, days		Seedlings inoc., No.		Seedlings severely diseased, and dead			
						No.		%	
		W*	V**	W	V	W	V	W	V
Radish (Scarlet Globe)	IS1	16	16	22	25	13	20	59	80
	IIS2	38	38	25	25	25	4	100	16
	IVS4A	33	33	25	25	25	22	100	88
	IIIS4C	35	35	25	25	25	9	100	36
	IIS6	33	33	25	25	25	8	100	32
Stock (Ten Weeks)	IS1	20	20	13	15	6	14	46	93
	IIS2	42	42	15	15	15	4	100	26
	IVS4A	39	39	14	14	14	7	100	50
	IIIS4C	37	37	13	13	10	10	91	91
	IIS6	37	37	14	14	14	0	100	0
Stock (Brompton)	IS1	26	26	8	6	1	2	12	33
	IIS2	48	48	4	5	4	0	100	0
	IVS4A	45	45	5	5	5	5	100	100
	IIIS4C	43	43	5	9	3	3	60	33
	IIS6	43	43	5	10	5	2	100	20
Wallflower (Finest Double Mixed)	IS1	20	14	21	20	16	17	76	85
	IIS2	20	14	24	32	24	1	100	2
	IVS4A	20	14	23	23	22	9	96	39
	IIIS4C	20	14	22	23	14	11	64	48
	IIS6	20	14	26	24	21	9	81	38
Lettuce (New York Special No. 12)	IS1	24	24	19	21	0	0	0	0
	IIS2	46	46	22	20	6	3	27	15
	IVS4A	43	43	19	19	6	5	32	26
	IIIS4C	41	41	19	21	7	1	36	5
	IIS6	41	41	18	17	6	1	33	6

* W = *wild type strain.*
** V = *cultural variant derived from the respective wild type.*

Although conditions in the seedling inoculations were specially conducive to infection, the pathogenicity of the strains on all hosts generally agreed with that on the potted stocks. The sensitivity of the test, however, may have been responsible for the fact that the variants from wild types IS1 and IIIS4C either killed or severely infected 93% and 91%, respectively, of the seedlings of Ten Weeks stock, although they had produced only a mild infection in the inoculations on older plants of this variety. The only other exception to close agreement between the two inoculation tests, also on Ten Weeks stocks, was the reverse of that above: the highly pathogenic variant from wild type

IVS4A was not as parasitic on the seedlings as on the older potted plants. On Brompton stocks, however, the virulence of these three variants was similar under both experimental conditions.



TEXT-FIG. 4. *The relative pathogenic vigor of five wild type strains (W) of A. Raphani and their cultural variants (V) on seedlings grown under aseptic conditions in test tubes.*

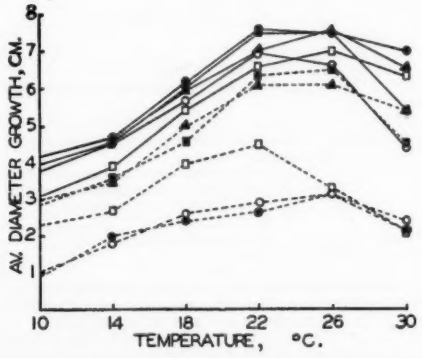
The weekly pathogenic strain IS1 and its variant failed to infect lettuce. The four others produced only slight lesioning and their variants were still less infective, except the one from IVS4A. On the other hosts, too, this variant was usually as parasitic as the virulent wild type from which it arose.

The results from these experiments demonstrate the existence in nature of pathogenic strains of *A. Raphani* and also show that cultural variation has widely divergent effects.

D. Factors Affecting Growth and Sporulation of the Wild Type Strains and Their Cultural Variants

1. *Temperature Relations*

The effect of temperature on diameter growth in culture of the five selected wild types and their variants was determined in the following experiment. Duplicate plates of potato dextrose agar were inoculated with each strain by means of 1-mm. discs from the margin of 10-day-old plate cultures. These had been inoculated from 10- to 17-day-old slant cultures of monohyphal-tip isolates. After 24 hr. at room temperature, the inoculated plates were placed in the cans of Wisconsin temperature tanks at 10°, 14°, 18°, 22°, 26°, and 30° C. The air temperature in the cans, which were covered with paper, practically coincided with these tank temperatures. Text-fig. 5 records the average diameters of the colonies after seven days.

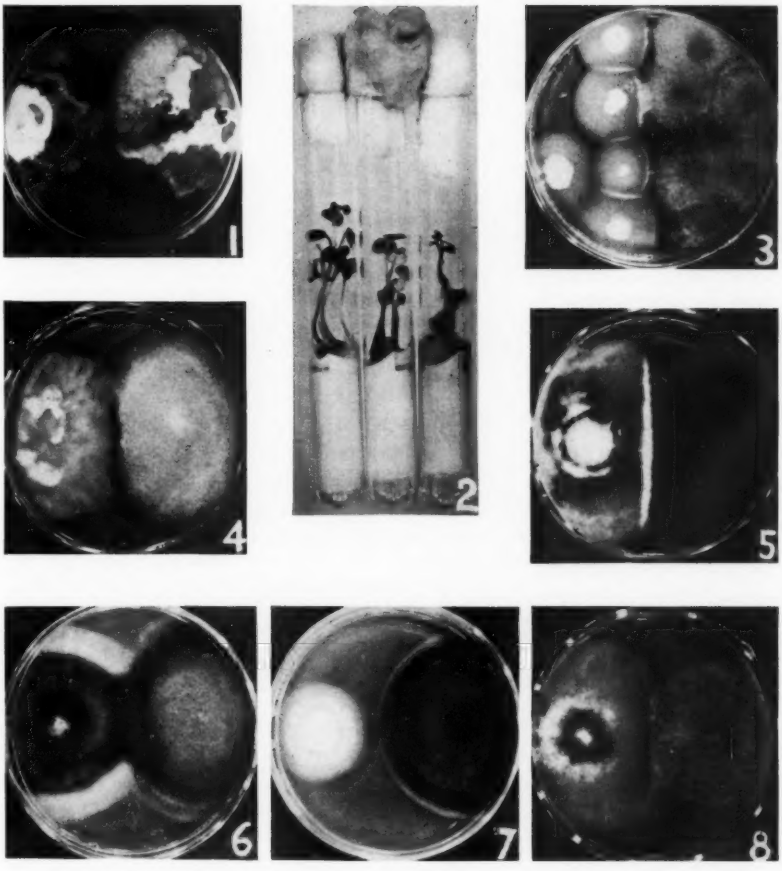


Text-fig. 5. *The effect of temperature, at seven days, on radial growth of five wild type strains of A. Raphani and their cultural variants.* ○———○ *Wild type IS1.* ○- - - -○ *Variant.* ■———■ *Wild type IS2.* ■- - - -■ *Variant.* ▲———▲ *Wild type. IVS4A.* ▲- - - -▲ *Variant.* □———□ *Wild type IIIS4C.* □- - - -□ *Variant.* ●———● *Wild type IIS6.* ●- - - -● *Variant.*

Below 30° C., all wild types, except possibly IIIS4C, showed approximately equal rates of radial growth. In contrast, their variants exhibited widely differing growth rates, but all grew more slowly than the wild types. The variants from IVS4A and IS2 grew the most rapidly and were only slightly slower than the wild types. Those from IS1 and IIS6, with practically equal rates, were the slowest growing of all strains. The remaining variant, from IIIS4C, was intermediate in growth rate between the two pairs mentioned.

The optimum temperature for growth of all strains evidently was between 22° and 26° C. The wild type strains and the two rapidly growing variants were highly sensitive to temperature changes, growth dropping sharply on each side of this peak. The three slowest variants, however, were much less responsive, their increase in growth from 10° C. to 22° and 26° C. being relatively gradual.

PLATE III



Wild type strains of A. Raphani and their cultural variants. FIG. 1. *Appearance of white, appressed variant in a monosporous wild type isolate after 10 months on an agar slant.* FIG. 2. *Pathogenicity of a wild type strain and its cultural variant on radish seedlings under aseptic conditions—control (left tube), cultural variant (center tube), wild type strain (right tube).* FIG. 3. *Monohyphal-lip isolates of a wild type strain (right half) and its variant (left half) which arose in a soil tube culture.* FIGS. 4 TO 8. *Five wild type strains (right colonies) and their respective variants (left colonies) which arose from monosporous isolates of the wild type strains.* FIG. 4. *Wild type IS1 and its variant;* FIG. 5. *Wild type IIS2 and its variant;* FIG. 6. *Wild type IVS4A and its variant;* FIG. 7. *Wild type IIIS4C and its variant.* FIG. 8. *Wild type IIS6 and its variant.*

Apparently cultural variation of three of the wild types studied resulted in a greatly reduced rate of growth, which was associated with an appreciable decline in response to temperature changes. On the other hand, cultural variation of two others resulted in only slightly less rapid growth and no loss of sensitivity to temperature.

2. *Survival in Dry Soil Culture*

The viability of the five wild types and their cultural variants for long periods in tubes of sterilized soil was demonstrated in the following experiment. A 15-month-old and an 18-month-old set of soil cultures, kept at room temperature and which had dried out after from six to eight weeks, were transferred to plates of potato dextrose agar by scattering soil grains over the medium. In three successive transfers, all cultures proved viable except the 18-month-old one of the variant from IS1. The fact that no hyphae were visible in this culture under the dissecting microscope indicated that, for some reason, the inoculum did not grow. The viability of the 15-month-old tube of this variant proved, however, that it, too, could survive long periods in dry sterile soil.

The survival of the wild types and their cultural variants for at least 18 months in dry soil cultures suggests a method for culturing *A. Raphani* and possibly other seed-borne fungi for long periods without the necessity of transfers. Furthermore, the greater stability of *A. Raphani* in soil compared to agar cultures suggests that possibly soil tube cultures could be used with the same advantage in the study of other seed-borne *Alternaria* species.

3. *Virulence after Prolonged Soil Culture*

Seedlings of radish var. Scarlet Globe grown under aseptic conditions in test tubes, as previously described, were inoculated at five days with five-day-old, potato-dextrose-agar plate transfers from 18-month-old soil cultures of the five selected wild types and their cultural variants. The 15-month-old soil culture of the variant from IS1 was used, as the 18-month-old one proved nonviable. Replicates of eight tubes of seedlings were inoculated with each of the strains studied. In addition, eight were inoculated with sterile agar, to serve as controls.

Table V records the results 13 days after inoculation at which time the control seedlings were still green and healthy.

A comparison of these results with those in Table IV proves that after 18 months in sterile soil the relative virulence of the wild types and their variants had remained essentially the same as that manifested under the same conditions after only from 16 to 38 days in agar culture. Pl. III, Fig. 2 shows seedlings killed by the wild type IIS6 but only slightly attacked by its cultural variant.

The conclusion is reached that *A. Raphani* may be cultured for long periods in dry sterile soil without loss of virulence.

TABLE V

VIRULENCE OF WILD TYPE STRAINS OF *A. Raphani* AND THEIR CULTURAL VARIANTS AFTER 18-MONTHS' CULTURE IN DRY STERILE SOIL

Strain	Seedlings inoc., no.		Seedlings severely diseased, and dead			
			No.		%	
	W**	V**	W	V	W	V
IS1*	38	35	13	21	34	60
IS2	38	35	38	11	100	31
IVS4A	38	39	38	38	100	97
IIIS4C	39	33	18	10	46	30
IIS6	36	37	36	11	100	30

* *The soil culture of the variant from IS1 was 15 months old.*
** *See footnote Table IV.*

4. *Traumatic Sporulation*

A. Raphani sporulates abundantly on infected host parts but, under ordinary conditions of agar culture, most isolates studied produced either no spores or only a few, mostly small atypical ones. Growth on agar containing infusions of radish leaves or table radish "roots" failed to stimulate abundant spore formation.

Accordingly, the wounding technique of Rands (9) was adopted, namely, the scraping of the surface of a 10- to 14-day-old potato-dextrose-agar plate culture which was then cut in a crisscross pattern into numerous small discs. After the treated cultures were left for an overnight period at room temperature, with the covers removed to allow partial drying, the three virulent wild types and the moderately pathogenic one produced an abundance of large typical spores. It should be noted that one of the virulent strains, IS2, did produce in normal cultures an appreciable number of spores but formed an even greater number under the stimulation of wounding. Groves (3) had similar success with this method when studying *Alternaria linicola* Groves and Skolko. The weakly pathogenic strain, however, formed exceedingly numerous chlamydospores but only a few small atypical conidia.

Wounded cultures with the covers on or under conditions of high humidity produced only a few small conidia. Unwounded control cultures either covered or uncovered produced relatively few or no spores.

In contrast, the wounding technique failed to stimulate sporulation in all five cultural variants, with the exception of an occasional undeveloped two- to three-celled spore. Evidently cultural variation of the wild types resulted in an almost complete loss of the capacity to sporulate in response to a traumatic stimulus.

5. *Traumatic Sporulation After Prolonged Soil Culture*

To determine the effect of prolonged soil culture on traumatic sporulation of *A. Raphani*, the 18-month-old soil cultures of the wild types and their variants were transferred to plates of potato dextrose agar. The resultant colonies were wounded in the manner previously described. The sporulation response of all strains was essentially similar to that prior to their prolonged culture in the tubes of sterile soil. The capacity of one of the wild types to form appreciable numbers of spores in normal cultures was also retained. As in the earlier test, none of the culturally derived variants produced spores or chlamydospores except those of IS1 and IIIS4C, which formed only a very few small undeveloped conidia.

These results prove that the sporulating capacity of *A. Raphani* in response to a traumatic stimulus is retained for a considerable period by dry soil cultures. Furthermore, the ability of certain strains to form spores on transfer to potato dextrose agar is also retained after soil culture.

6. *Effect of Constituents of Radish on Sporulation*

As most isolates of *A. Raphani* produce relatively few or no spores in ordinary potato-dextrose- or malt-agar cultures but sporulate abundantly on diseased radish tissue, it follows that the host plant contains one or more substances essential for sporulation. Whether these are organic or inorganic was next investigated.

(*a*) *Heat-stable Constituents*

To determine the possible role of heat-stable constituents in sporulation, radish seedlings were grown under aseptic conditions in test tubes, as described earlier, and killed after six days by autoclaving for 20 min. at 15 lb. pressure. After cooling, they were inoculated in triplicate with plate transfers of the 18-month-old soil cultures of the five wild types and their variants. A duplicate test on autoclaved seedlings was conducted, and gave similar results. Sporulation on the radish seedlings in the pathogenicity test of the 18-month-old soil cultures served as a control. Table VI shows the relative production, after 14 to 18 days, of both spores and chlamydospores.

All five wild types sporulated about as abundantly on seedlings killed by autoclaving as on those parasitized by the fungus. Although the three virulent strains and the moderately pathogenic strain produced mostly large typical spores, the weakly infective one formed many small atypical conidia. The latter strain also produced abundant chlamydospores on all seedlings, whereas the others formed relatively few.

Four of the five cultural variants formed very few or no spores either on the parasitized or on the autoclaved seedlings. Under both conditions, however, the variant from the weakly pathogenic strain produced abundant, although mostly small, conidia. It should be remembered, however, that this variant, which was slightly more pathogenic than its weak wild type, was similar to the others in forming few or no spores in wounded or normal agar cultures.

TABLE VI

SPORULATION AND CHLAMYDOSPORE PRODUCTION OF WILD TYPE STRAINS OF *A. Raphani* AND THEIR CULTURAL VARIANTS ON PARASITIZED AND AUTOCLAVED RADISH SEEDLINGS

Strain		Parasitized seedlings		Autoclaved seedlings	
		Spores	Chlamydospores	Spores	Chlamydospores
IS1	W*	4**	3**	4	5
	V*	4	0	4	0
IS2	W	5	0	5	1
	V	0	0	0	0
IVS4A	W	5	0	5	2
	V	1	1	0	0
IIIS4C	W	3	1	5	1
	V	2	0	2	0
IIS6	W	5	0	5	3
	V	0	0	0	0

* *See footnotes Table IV.*

** *1 = trace of spores and chlamydospores; 2 = relatively few; 3 = moderate number; 4 = abundant; 5 = extremely abundant.*

Evidently the radish seedlings contained one or more substances essential for abundant sporulation of wild type strains of *A. Raphani.* These substances are obviously heat stable as sporulation was equally abundant on the autoclaved as on the parasitized seedlings. Therefore, to determine whether they were organic or inorganic, the effect on sporulation of the ash, or mineral, fraction of radish was next investigated.

(*b*) *Mineral Constituents*

As relatively few spores were formed on ordinary potato dextrose agar, the growing of the pathogen on this medium supplemented with the ash of radish should indicate whether the mineral or the organic content of the host was responsible for abundant sporulation on the seedlings. As the seedlings had been grown in distilled water, their mineral content was similar to that of the seeds. Accordingly, the procedure was simplified by ashing radish seed from the same sample rather than the seedlings. The ash obtained was pale whitish-gray, and a solution of it in distilled water had a reaction of pH 7.4.

Potato dextrose agar was supplemented with the ash at the rate of 2 gm. per 500 cc., the reaction of which was adjusted from pH 6.8 to pH 6.0 to equal that in the ordinary agar medium. Plates of both kinds of potato dextrose agar were inoculated, in triplicate, with particles from approximately 19-month-old soil cultures of the five wild types employed in previous experiments. In addition, one plate of each kind of medium was similarly inoculated with their cultural variants, as one had produced abundant spores both on autoclaved and on parasitized seedlings.

The colonies that developed were examined after 12 days at room temperature. The addition of the ash to potato dextrose agar failed to stimulate

abundant sporulation in any of the strains. The wild types produced either no spores or very few on both media, except the strain IS2, which previously was shown to form an appreciable number on potato dextrose agar. None of the variants produced any spores on either medium.

These results support the conclusion, therefore, that the abundant sporulation of wild type strains of *A. Raphani* on the autoclaved and on the parasitized seedlings was due to one or more heat stable organic constituents and not to the mineral content of the plants.

7. *Nutrient Requirements*

To obtain some indication of the nutrient requirements of the five wild types and their variants, the method of Lochhead and Chase (4) was adopted. They used seven liquid media of increasing complexity as follows: (1) basal glucose-nitrate salts—Medium B; (2) amino acids—Medium A; (3) growth factors—Medium G; (4) amino acids and growth factors—Medium AG; (5) yeast extract—Medium Y; (6) soil extract— Medium S; (7) yeast and soil extracts—Medium YS. Proline was not available for the amino acid medium in the present experiment, otherwise all media were the same as those used by Lochhead and Chase. All media were autoclaved in 3 cc.-amounts in tubes 10 cm. long and 1.3 cm. wide. Transfers from 18-day-old potato-dextrose-agar plates derived from 19-month-old soil cultures were used to inoculate the various media. In the absence of spores, small portions of aerial hyphae free of agar were picked off the plate colonies and macerated in sterile distilled water on a flamed slide. Each tube of medium was inoculated with one loopful of the hyphal-fragment suspension by means of a 1 mm.-loop. Each medium was inoculated with the five wild types in replicates of five and with their variants in replicates of four.

After 10 days at room temperature, the relative amounts of growth, sporulation, and chlamydospore production were recorded on the basis of a numerical rating and are presented in Table VII. Growth response was based on the relative amounts of mycelium produced, whereas the rating of conidial and chlamydospore production was based on microscopic examination. The numbers 1 to 4 were assigned to indicate increasing growth response, but only a difference of two was considered significant. Similarly, the numbers 1 to 5 were assigned to indicate increasing numbers of spores and chlamydospores, as previously used to indicate sporulation on radish seedlings (Table VI).

(*a*) *Growth*

Growth of the wild types in the various media consisted of thick surface mats of dark olive-gray mycelium with relatively small amounts of colorless submerged hyphae. The strain IIIS4C produced, however, only a small amount of pigment in the basal and amino acid media and was almost colorless in the soil extract medium.

All cultural variants also produced in most media thick surface mats of mycelium, either entirely colorless, or, if slightly pigmented, only in the yeast extract and in the yeast plus soil extract media. The reaction of the media

TABLE VII

NUTRIENT REQUIREMENTS OF WILD TYPE STRAINS OF *A. Raphani* AND THEIR CULTURAL VARIANTS

Strain		Growth							Sporulation							Chlamydospore production						
		B*	A	G	AG	Y	S	YS	B	A	G	AG	Y	S	YS	B	A	G	AG	Y	S	YS
IS1	W**	4†	4	4	4	4	3	4	0†	2	2	2	2	0	2	4‡	4	4	4	4	4	4
	V**	4	4	4	4	4	2	4	0	1	1	1	3	0	3	0	1	1	1	3	0	3
IS2	W	4	4	4	4	4	3	4	3	3	3	4	5	0	2	3	3	3	3	3	5	4
	V	3	4	4	4	4	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IVS4A	W	4	4	4	4	4	4	4	2	3	3	4	5	1	1	2	3	3	4	3	5	4
	V	3	3	3	3	4	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IIIS4C	W	4	4	4	4	4	4	4	0	1	1	2	2	1	1	1	0	0	0	1	2	1
	V	2	4	2	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IIS6	W	4	4	4	4	4	4	4	2	3	3	4	5	1	1	3	2	2	2	2	5	5
	V	2	2	2	2	4	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* *See text for the kinds of media.*
** *See footnotes Table IV.*
† *See text for rating.*
‡ *See footnote Table VI for rating.*

showed only negligible change from the original pH 6.8 due to growth of the various strains.

The data in Table VII show that the five wild type strains grew equally well on all seven media, indicating they could produce maximum growth on a simple medium containing glucose, nitrate, and the usual salts required by microorganisms.

Three of the variants showed a similar, nonspecific growth response. In contrast, the two remaining variants showed special nutrient requirements. The variant from IIIS4C required amino acids, whereas that from IIS6 required unknown substances in yeast or soil extracts. The ability of the variant from IIIS4C to produce maximum growth in yeast and soil extracts indicates that these extracts contained the amino acids present in the synthetic amino acid medium or substituent materials.

These results are interpreted to mean that the cultural variant of wild type IIIS4C was less able than the wild type to synthesize prerequisite amino acids from a simple glucose-nitrate-salts medium. The cultural variant of the wild type IIS6, however, almost entirely lacked the capacity to synthesize, not only the amino acids in the synthetic medium, but additional ones and possibly growth factors present in yeast and soil extracts.

(*b*) *Sporulation*

In contrast to the vigorous growth of the wild type strains in the simple basal medium, none of the strains produced more than a few spores, except IS2. This was the only strain which produced appreciable numbers of spores

in ordinary potato-dextrose-agar culture. The three virulent wild types produced abundant spores in the amino acid plus growth factor medium but showed maximum sporulation in yeast extract. The two weaker strains IS1 and IIIS4C produced only a few spores, even in yeast extract. It should be recalled that these strains produced abundant spores on parasitized and autoclaved radish seedlings. Evidently the substances they require for abundant sporulation are present in the seedlings but not in yeast extract. None of the wild types produced more than an occasional spore in soil extract or in soil extract plus yeast extract. As sporulation in the latter medium was also poor, the lack of spores in soil extract was not due to a lack of nutrients but rather to the presence of inhibiting substances.

Four of the five cultural variants failed to produce spores in any of the seven media used. The variant from the weakly pathogenic strain, however, formed a moderate number in yeast extract. This variant, therefore, was similar to the wild types, as it also formed the most spores, although in greatly reduced numbers, in yeast extract. It did produce more spores, however, than the weakly pathogenic strain from which it was derived and compared to which it was previously shown to be slightly more virulent. In addition, sporulation of this variant, like that of the wild types, was suppressed in soil extract but, unlike that of the latter, was not suppressed in soil extract supplemented with yeast extract.

In the hope of determining what substances in the yeast extract medium might account for the even greater sporulation than that in the amino acid plus growth factor medium, analytical data of the yeast extract used were obtained from the company manufacturing the preparation. Through the courtesy of the Director of the bacteriological laboratories of Difco Laboratories Incorporated, Detroit, Mich., a typical analysis of their "Bacto-Yeast" extract preparations was obtained, and is as follows:

Total nitrogen	11%	*Lysine	4.0%
Ammonia nitrogen	0.4%	Methionine	0.79%
Heat coagulable nitrogen	Nil	Phenylalanine	2.2%
Proteose nitrogen	Nil	Threonine	3.4%
Cystine and *Cysteine	4.3%	Tryptophan	0.88%
Van Slyke nitrogen	6.0%	Tyrosine	0.60%
Ash	12.0%	Valine	3.4%
*Riboflavin	43.0 μgm. per gm.	Chloride	0.19%
*Thiamine	0.05–9.6 μgm. per gm	Sulphur	1.39%
*Nicotinic acid	182.0 μgm. per gm.	Lead	16.0 p.p.m.
*Pyridoxine	23.2 μgm. per gm.	Arsenic	0.11 p.p.m.
*Pantothenic acid	130–263.0 μgm. per gm.	Manganese	7.8 p.p.m.
Folic acid	3.4–10.0 μgm. per gm.	Zinc	88.0 p.p.m
*Biotin	0.06–1.0 μgm. per gm.	Phosphorus	0.89%
p-Aminobenzoic acid	38–120.0 μgm. per gm.	Iron	0.028%
*Arginine	0.78%	Copper	19.0 p.p.m.
*Aspartic acid	5.1%	SiO_2	0.052%
*Glutamic acid	6.5%	Potassium	0.042%
*Glycine	2.4%	Sodium	0.32%
Histidine	0.94%	Magnesium	0.030%
Isoleucine	2.9%	Calcium	0.0406%
*Leucine	3.6%		

The inorganic constituents of the amino acid plus growth factor medium and "Bacto-Yeast" extract are similar, as the trace metals lead, arsenic, manganese, zinc, and copper, as well as silicon dioxide, undoubtedly also occurred as contaminating substances in the salts of the basal medium. Thirteen of the 16 amino acids and growth factors in the amino acid plus growth factor medium are also present in "Bacto-Yeast" extract and are marked with an asterisk in the analysis list. Additional compounds present in the yeast extract but not in the amino acid plus growth factor medium are as follows: cystine, folic acid, *p*-aminobenzoic acid, histidine, isoleucine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine—all amino acids except folic acid, a vitamin.

Obviously, one or several of these amino acids, or folic acid, in addition to one or more of the starred amino acids and growth factors accounted for the profuse sporulation of the wild types of *A. Raphani* in yeast extract. The possibility exists that the abundant sporulation in the wounded plate cultures may have been due to the formation of these essential compounds by means of a traumatic hormone.

8. *Chlamydospore Production*

Maximum production of chlamydospores by the three virulent wild types occurred only in the soil extract and in the yeast extract plus soil extract media, whereas that in the weakly pathogenic strain was equally abundant in all media. In contrast, the moderately pathogenic strain either failed to form chlamydospores or produced only a few in some of the nutrients, which parallels its equally poor sporulation. It is interesting to note that, although sporulation in all strains was suppressed in soil extract, chlamydospore production was greatest in this medium. Such contrasting effects probably represent a reaction to staling products resulting from the type of metabolism initiated in soil extract.

The only variant to form appreciable numbers of chlamydospores was that from the weakly pathogenic strain IS1, most being produced in the yeast extract and in the yeast extract plus soil extract media. The only other that formed chlamydospores was the strongly pathogenic variant from IVS4A, which produced only a few, also in these media. Further, although soil extract supported maximum production of chlamydospores in the five wild types studied, this medium suppressed their formation in the two variants mentioned.

9. *Sporulation on Complex Agar Media*

The profuse sporulation of the virulent wild types in yeast extract solution suggested that *A. Raphani* might also produce abundant spores on potato dextrose agar supplemented with the extract. In this connection, the value of yeast extract as well as that of dehydrated beef bile was investigated.

Accordingly, two lots of potato dextrose agar, one lot supplemented with "Bacto-Yeast" extract and the other with "Bacto-Oxgall", a dehydrated fresh beef bile preparation, were made up at a concentration of 2 gm. of extract

per liter. After autoclaving, both media had a reaction of pH 6.0, the same as that of ordinary potato dextrose agar. Plates of both kinds of media were inoculated with a few particles from approximately 20-month-old soil cultures of the five wild types and their variants previously studied.

After 10 days at room temperature, the three virulent wild types had produced extremely abundant spores, the moderately pathogenic strain only a few, and the weakly infective strain none.

None of the variants produced any spores or chlamydospores on either medium. It should be noted that the variant from IS1 had formed a moderate number of spores in the liquid yeast extract medium. Evidently this variant was not able to utilize the amino acids and growth factors essential for sporulation present in yeast extract when the latter was combined with agar. This inability may be related to the colloidal properties of agar, which possibly rendered essential substances unavailable to the variant. It follows that the wild types were able to circumvent any such effect of agar colloids.

The abundant sporulation of the virulent wild types on potato dextrose agar supplemented with "Bacto-Oxgall" indicates that the essential amino acids and growth factors present in yeast extract are also present in the dehydrated beef bile preparation. That the bile extract evidently contained an inhibiting factor as well was apparent, as all strains tested grew more slowly on potato dextrose agar containing the extract than on the ordinary agar medium or on that containing yeast extract. At 10 days, the diameter of most of the wild types on ordinary potato dextrose agar was 9.0 cm., but was only from 4.5 to 5 cm. when this medium contained "Bacto-Oxgall."

Control by Seed Treatments

Seed treatments reported to date for the control of *A. Raphani* in radish seed have proved of little value. Groves and Skolko (3) found that, when infected seed was treated with Ceresan, Semesan, Arasan, and Spergon and plated on agar, the percentage developing *A. Raphani* was only slightly reduced. McLean (6) considers *A. Raphani* to be the principal cause of low germination of radish seed in Michigan. Treatment of such seed with the common dusts failed to increase germination. He reported also that, although hot water treatment at 50° C. for 25 min. killed the pathogen, this procedure failed to increase germination. Neergaard (8) found that the degree of control of seed-borne *Alternaria* and *Stemphylium* species in different lots of the same kind of seed varied considerably. He did not report any experiments with *A. Raphani*. This may explain why, in contrast to the results of McLean, a substantial increase in germination and decrease in infected seedlings was obtained in the seed treatment experiments reported below.

The results of these experiments both in the greenhouse and in the field are given in Tables VIII and IX. The fungicides used—Arasan, 1% Ceresan, and Spergon—were applied in the form of dusts, the excess being removed by sifting through cheesecloth prior to planting the seed. In the greenhouse

TABLE VIII

RESULTS OF SEED TREATMENT TESTS IN THE GREENHOUSE FOR THE CONTROL OF SEEDLING DISEASE IN RADISH DUE TO *A. Raphani*

Variety	Treatment	Emergence, %	Post-emergence blight, %	Total disease, %	Reduction in disease, %	Fresh weight, gm.
Sparkler	Untreated	79.6	5.0	31.1	—	28.7
	Arasan	96.0	3.1	12.5	18.5	42.5
	Spergon	96.0	3.1	15.3	15.8	43.7
	1% Ceresan	95.3	0.0	4.9	25.2	49.0
Scarlet Globe	Untreated	77.0	11.8	50.1	—	28.7
	Arasan	91.0	4.4	17.9	32.2	39.1
	Spergon	83.0	6.1	23.6	26.5	30.9
	1% Ceresan	91.0	4.4	9.9	40.2	34.0

TABLE IX

RESULTS OF SEED TREATMENT TESTS IN THE FIELD FOR THE CONTROL OF SEEDLING INFECTION IN RADISH VAR. SCARLET GLOBE DUE TO *A. Raphani*

Treatment	Emergence, %	Disease, %	Reduction in disease, %
Untreated	57	56	—
Arasan	71	20	36
Spergon	62	32	24
1% Ceresan	54	33	23

tests, the seed was planted in a sand–loam mixture that had been partially sterilized at 5 lb. steam pressure for five hours one month previous to use. High midsummer temperatures prevailed in the greenhouse during the test, often over 90° F. on the hottest days. In the field, with the exception of two days, maximum temperatures ranged from 70° to 89° F., and minimum temperatures from 50° to 70° F. Rainfall during the experimental period consisted of two precipitations of 0.7 and 0.25 in.

A. Greenhouse Tests

It is evident from the results in Table VIII that, in the greenhouse tests, all treatments appreciably reduced seedling infection—1% Ceresan being the most and Spergon the least effective under the conditions present. It should be noted that the lessened effectiveness of the treatments in the Sparkler variety is largely due to the low disease incidence in one of the control replicates with the resultant lowering of the average. All treatments increased emergence by approximately 15% and also resulted in greater fresh weights of the seedlings.

B. Field Tests

As in the greenhouse tests, all treatments reduced seedling infection in the field tests. In contrast, however, to the superior value of 1% Ceresan in the greenhouse tests, Arasan proved the most effective in the field. Only Arasan appreciably increased emergence, whereas 1% Ceresan had no effect.

These differences between results of the greenhouse and field tests may have been related to soil temperatures, but unfortunately these were not recorded as such effects were not anticipated.

Discussion

A number of *Alternaria* species are able to infect and establish a dormant mycelium within the seed of cruciferous vegetables. The relationship between the developmental stages of the seed and infection and extent of colonization by these fungi has received little or no attention. About all that can be said at present is that they are capable of attacking young green siliques, and presumably can also establish themselves within the green immature seeds at that time. The extent to which the pathogens subsequently colonize the seeds will depend on environmental factors affecting both host and fungi, not only during the maturation and ripening process, but also during the period of curing and storage. Evidence presented in the present paper shows that *A. Raphani* is capable of invading all parts of infected radish seed. This explains why, in seed samples carrying a high percentage of the fungus, many seeds fail to germinate and many seedlings succumb to pre- or postemergence blight.

If soil environment plays a role in determining the severity of seedling infection in seed-borne diseases, the view is held that the most critical effects of this factor probably would be exerted during the early pre-emergent stages of host development. Evidence obtained in the present investigation indicated that both soil temperature and moisture markedly affected the incidence and severity of seedling infection due to *A. Raphani*. The results obtained were considered to be a function of the effect of these factors both on the host and on the pathogen. Why the soil temperature effect was apparent only when severely infected seed was sown is obscure and awaits further research for clarification. The point may be raised here as to whether or not this also holds true in seedling infection caused by other seed-borne *Alternaria* species, such as *A. Brassicae* (Berk.) Sacc., *A. oleracea* Milbr., *A. circinans* (B. & C.) Bolle, *A. radicina* Meier, Drechsler, & Eddy, *A. Dauci* (Kühn) n. comb., and *A. linicola* Groves & Skolko.

Although infected plant parts in or on the soil may serve to spread seed-borne *Alternaria* species during any current season, it is not clear that an overwintering soil-borne inoculum is established in this manner. Such evidence as was obtained in the present studies discounts this possibility for *A. Raphani* at least. If this is generally true, *A. Raphani* is not potentially as serious as certain seed-borne cereal pathogens, such as *Helminthosporium sativum* P.K. and B., *Fusarium graminearum* Schwabe, *F. culmorum* (W. G. Smith) Sacc.,

and *F. avenaceum* (Fr.) Sacc., which do have a saprophytic cycle in the soil. Apparently *A. Raphani* is not able to compete successfully with the microorganisms of the soil but is rather specifically adapted to invade and survive in the seed of its host.

The severe leaf blight of stocks and wallflowers resulting from inoculation with *A. Raphani* suggests that plantings of these flowers, either in the field or in the greenhouse, should be some distance from radish crops which might harbor the fungus, especially as it is not uncommon in radish seed from all parts of Canada and from many areas in the United States and Europe.

The demonstration that naturally occurring biotypes of *A. Raphani*, although closely related culturally, may show considerable diversity in virulence, raises the question as to whether or not a similar diversity exists in other seed-borne *Alternaria* species on crucifers. The failure of Groves and Skolko's (3) isolates of *A. Raphani* to attack seedlings of wallflower and their weak attack on cabbage, suggest the possibility that, by chance, a weakly pathogenic strain was used to inoculate these hosts.

The fact that *A. Raphani* readily produced cultural variants, some of which were markedly less pathogenic than the naturally occurring, or wild type strains, shows how stock cultures not purified regularly by monosporous isolations might eventually become attenuated. This evidently was the underlying reason for Neergaard's (8) observation that most of his isolates of *A. Raphani* showed "a distinct decrease in virulence in the latter part of the experimental series", the whole of which covered a 3-year period. Evidence was obtained, however, that variation did not always result in a loss of virulence and might even result in an increase. Similar observations were also noted in *H. sativum* by Christensen (2) who, in reference to the mutant strains he had isolated from sectoring colonies, states that "most were like the parent form but two were decidedly more virulent than their respective parents."

The spontaneous origin and stability of the cultural variants, with no sign of reversion to the wild types, favors the view that they are mutants. Furthermore, the study of cultural habit, pathogenicity, rate of growth, sporulating capacity, and nutrient requirements indicated that the effect of cultural variation on any one of these characteristics was independent of that on any other. This kind of variation corresponds to that arising as a result of mutation. It is interesting to note that Ullstrup (10), in his study of "normal" and variant cultures of *Glomerella Gossypii* (South.) Edg., found that the only cultural characteristic related to pathogenicity was that of growth rate. Finally, in two of the variants described here, the loss of ability to perform certain biochemical syntheses necessary for growth, parallels the behavior of artificially induced mutants of *Neurospora crassa* Shear & Dodge. Genetic studies of such mutants have been possible in *Neurospora* owing to its having a sexual stage and have led Bonner (1) to postulate a theory of genic control

of specific cellular reactions. All these considerations support the view that the cultural variations noted in the present study of *A. Raphani* were brought about by mutation.

The survival of *A. Raphani* in dry sterile soil cultures, and its high degree of stability in this environment, suggest that this method may be applied with obvious advantages to the culture and study of other seed-borne fungi that also mutate readily on synthetic agar media.

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NORTH AMERICAN SPECIES OF *CHRYSOMYXA*[1]

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Abstract

The adaptation of the genus *Chrysomyxa* to a short growing season is discussed. Keys are given to the aecial and to the uredinial or telial stages of species that occur in North America. Range extensions are given, particularly for northern Canada. *C. Rhododendri*, newly reported in North America, and *C. Cassandrae* are shown to be barely distinguishable from *C. Ledi* and are made varieties of it. *C. Woronini* is reported for the first time in North America.

Introduction

Extensive botanical work in northern Canada by members of the Division of Botany and Plant Pathology has recently yielded numerous collections of rusts in the genus *Chrysomyxa*, and has allowed a fuller understanding of their biology, importance, and distribution.

The genus *Chrysomyxa* is singularly adapted to the short growing season of the subarctic. In the typical, long-cycled species, with aecia (of the form genus *Peridermium*) on *Picea* and with uredinia and telia on various dicotyledonous evergreens, the sexual and uredinial phases of the life cycle occur simultaneously and more or less independently, in sharp contrast to the situation in many rust genera in the temperate zone. This condition is possible by virtue of restriction to evergreen plants as uredinial and telial hosts. The aeciospores are usually shed in late summer, and both they and the late crops of urediniospores form overwintering dikaryotic mycelia in the current season's leaves. In the spring these mycelia produce uredinia and telia simultaneously, sometimes in the same sorus. Thus, while the urediniospores are propagating the rust upon the new leaves of the dicotyledonous host, the basidiospores are infecting the new needles or cones of the spruce. The aecial stage is, of course, of value in allowing gene recombination and also helps to propagate the rust; but it is not essential to the persistence and spread of those species that possess urediniospores, some of which are found far beyond tree line.

The aim of the present paper is to discuss the taxonomy of North American species and the distribution of Canadian species; to present a key to the aecial stages, the lack of which has hindered the understanding of the genus; and to present a new key to the uredinial and telial stages, with emphasis on morphology rather than on host relationship. It is first necessary to consider some of the diagnostic characters that must be used in separating certain species. Inadequate evaluation of these characters has led in the past to the suggestion of apparent distinctions and the concealment of real ones.

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The range of widths and lengths of aeciospores and urediniospores is, of course, of value in *Chrysomyxa* as in almost all rusts, but the spores of some species are so variable that these characters must be determined carefully. The measurement of about ten randomly selected spores is inadequate, often suggesting large differences between collections that are actually identical. The writer has found it advisable to search for extremes of length and width and then fill in the gaps to ensure that a smooth series can be obtained. Figures that fall far outside the continuous range are put in parentheses in the summary of data for the specimen.

Spore wall thickness and wall ornamentation are usually valuable and may allow the distinction, from single aeciospores, of two otherwise indistinguishable peridermia. It is essential that examination of these characters be made with the oil-immersion objective. Use of the 4 mm. objective often causes too high a figure to be given for wall thickness, partly because of diffraction fringes and partly because there is often an unpigmented layer of cytoplasm adjacent to and simulating the hyaline wall. The oil-immersion objective is essential to the study of wall markings; it allows faster and more accurate measurement of spore sizes; and, by elimination of glare, gives a far more brilliant image and much less eye strain than a high-powered dry objective with its serious external reflections. The characterization of wall markings as low or high, fine or coarse is of limited value. It is sometimes necessary to record the range of heights, widths, and spacings of warts, disregarding any obvious abnormalities. Spacing, as used in this paper, refers to the range of distances from center to center of two adjacent warts, not to the gap between them.

An important characteristic of the genus *Chrysomyxa* is the marked tendency for resemblance both of aeciospores to urediniospores of the same species and of habit in the alternate hosts. Thus *C. Pyrolae*, which is systemic in its dicotyledonous host and has urediniospores with large, irregular warts, is systemic in the cones of *Picea* and has aeciospores with large, irregular warts. This characteristic allows one to prognosticate with some confidence the appearance of undiscovered aecial stages or the relationship of unidentified ones.

Key to North American Species

In the key given below to the aecial stages, it should be noted that the three varieties of *C. Ledi*, hitherto treated as separate species, are considered indistinguishable. For the species whose aecia are still unknown, tentative provision has been made on the basis of presumed resemblance to the uredinia. The key to the uredinial and telial stages is based as fully as possible on morphology in order to emphasize the relationship of certain species. Dimensions given may be approximate where they cover more than one species.

KEY TO PERIDERMIA

1. Scattered on leaves of *Picea*, sometimes sparingly on cones
   2. Spores usually lanceolate, c. 70 × 20μ ..... *C. Piperiana*
   2. Spores globose to ellipsoid
      3. Spores large, 27–47(–62) × 19–34μ
         4. Wall, excluding warts, 1.5–2.8μ thick; warts 1.5–3.0μ high ..... *C. ledicola*
         4. Wall, excluding warts, 0.3–1.3μ thick; warts 1.0–2.2μ high ..... *C. Empetri*
         4. Wall, excluding warts, less than 0.5μ thick; spores slightly narrower than in last two species, 19–27μ wide ..... *C. roanensis*
      3. Spores small, 18–33 × 14–25μ
         5. Spore warts more or less uniform ..... *C. Ledi* vars.
         5. Unknown; warts probably lacking from part of spore ..... *C. ilicina*
         5. Known only from cultures; no adequate description of warts available ..... *C. Chiogenis*
1. More or less systemic in cones of *Picea*: warts often elongate ..... *C. Pyrolae*
1. Systemic in stunted shoots and leaves of *Picea* ..... *C. Woronini*

KEY TO UREDINIA AND TELIA

1. Uredinia present, usually conspicuous
   2. Uredinia localized; urediniospore warts usually more or less circular
      3. Urediniospores ellipsoid
         4. Urediniospores large, 25–45 × 20–34μ
            5. Epiphyllous; urediniospores to 34μ wide
               6. Urediniospore wall, excluding warts, 0.4–1.8μ; warts mostly stellate to slightly irregular, 1.0–3.0μ high, 0.4–2.0μ wide ..... *C. ledicola*
               6. Urediniospore wall, excluding warts, 0.3–1.0μ; warts cylindrical to slightly stellate, 0.7–2.2μ high, 0.3–1.0μ wide ..... *C. Empetri*
            5. Hypophyllous; urediniospores to 26μ wide ..... *C. roanensis*
         4. Urediniospores small, 19–34 × 13–22μ
            7. Warts more or less evenly distributed; on Ericaceae
               8. Warts mostly shallow, broad, polygonal, or elongate, 0.5–2.0μ wide, 0.8–5(7)μ long ..... *C. Ledi* var. *Ledi*
               8. Warts mostly cylindric, 0.3–1.0μ wide, occasionally irregular and up to 1.0 × 2.5μ ..... *C. Ledi* var. *Cassandrae*
               8. Warts cylindric, 0.3–1.0μ wide, rarely slightly irregular ..... *C. Ledi* var. *Rhododendri*
            7. Warts generally absent from part of spore; on Ilicaceae ..... *C. ilicina*
      3. Urediniospores lanceolate ..... *C. Piperiana*
   2. Uredinia systemic or partially so; urediniospore warts usually elongate
      9. Urediniospore warts entire ..... *C. Pyrolae*
      9. Urediniospore warts fluted ..... *C. Chiogenis*
1. Uredinia lacking
   10. Telia systemic, on *Ledum* leaves ..... *C. Woronini*
   10. Telia localized, on *Picea* leaves ..... *C. Weirii*
   10. Telia localized, on *Arctostaphylos* leaves ..... *C. Arctostaphyli*

Discussion of Species

The distributional data given below under the various species supplement those given by Arthur (1). The abbreviations Frank., Keew. and Mack. are used for the Districts of Franklin, Keewatin, and Mackenzie of the Northwest Territories. The abbreviations N. Man. and N. Que. are used to indicate the sparsely inhabited northern parts of Manitoba and Quebec, approximately

from James Bay northward. The abbreviation DAOM is employed for specimens in the mycological herbarium of the Division of Botany and Plant Pathology.

Chrysomyxa ledicola (Peck) Lagerh.

Chrysomyxa ledicola is perhaps the dominant rust of the Canadian boreal forest. It is common from coast to coast on both *Picea* and *Ledum*. In the more southerly spruce forests, *C. Ledi* var. *Ledi* and *C. Ledi* var. *Cassandrae* may be equally or even more abundant, but, as one progresses northward, *C. ledicola* largely replaces them. It has been found without difficulty in almost every area where *Ledum* is plentiful, and it is sometimes impossible to secure phanerogamic specimens free of it. It is sometimes extremely heavy on spruce over wide areas; for example, Riley (7) reported that much of the white spruce forest of eastern Manitoba was so heavily infected in 1948 that the discoloration was evident from the air. Such epidemics presumably have a serious effect on the following year's growth, as infected needles are generally shed late in their first season. Valuable field associations of this and the next species, found at Great Whale River, Que., in 1949, provided specimens (*C. ledicola* DAOM 23439 and 23440, *C. Empetri* DAOM 23436 and 23438, all on *Picea glauca*) that made possible the absolute distinction of these very similar species. Associations such as these are sometimes found in which a young spruce emerging from a mat of the telial host is completely covered by aecia for the lowest foot or so and almost free from infection higher up, and, if selected with adequate precautions, are as reliable as artificial inoculations. A detailed description of the aecial and uredinial stages follows. Aecia scattered, amphigenous or occasionally episquamous, occasionally mainly epiphyllous or mainly hypophyllous on a given tree, but often equally on all four faces of a needle; habit possibly dependent on conditions at time of infection. Aeciospores 27–46(–55) × 22–34(–38)μ; wall, excluding warts, 1.5–2.8μ, hyaline; warts truncate, slightly tapered, generally fluted and therefore stellate in surface view, 1.5–3.2μ high × 0.8–2.0μ wide × 1.5–3.0μ spacing. Uredinia scattered, epiphyllous. Urediniospores mostly 24–45 × 20–33μ; wall, excluding warts, 0.5–1.7μ hyaline; warts nearly cylindrical to stellate or irregular, 1.0–3.0μ high × 0.4–2.0μ wide × 1.0–3.2μ spacing. In most specimens neither aeciospores nor urediniospores show the full size range, the upper limit of length being particularly variable. Some of this variation is perhaps geographic. Only two collections examined, both from Anticosti I., Que., yielded aeciospores more than 46μ long or 34μ wide.

Specimens examined give the following range extensions: *Picea glauca*, Mack., Man., Ont., N. Que. *P. glauca* var. *albertiana*, Yukon. *P. mariana*, Ont., N. Que. *P. pungens*, Alta., Man., Que., P.E.I. *P. rubens*, Que. *Ledum groenlandicum*, Mack., N. Man., N. Que. *L. palustre* var. *decumbens*, Yukon, Mack., Frank., Keew., N. Man., N. Que., including stations far beyond limit of spruce.

Chrysomyxa Empetri (Pers.) Schroet.

Chrysomyxa Empetri is probably almost co-extensive with *Empetrum nigrum* in northern Canada, but the small leaves of the host make the rust inconspicuous and relatively few specimens are available. Close to tree line across the continent it is probably as important as *C. ledicola*. The relatively recent recognition by Faull (5) of the aecial stage and the paucity of aecial collections are partly explained by the resemblance of this stage to that of *C. ledicola*. If the situation at Great Whale River in 1949 is typical of the northern tree line generally, the aecial stage is common and abundant. *Empetrum nigrum* is such an important element of the tree-line flora that *C. Empetri* is to be ranked as a definite, though not a leading, member of the complex of factors that checks the advance of spruce into the barrens. Like *C. ledicola* this species evidently extends far beyond the limit of spruce, for it is recorded from Frobisher Bay, Baffin Is. The following description of the aecia and uredinia shows the resemblance to *C. ledicola*. Aecia scattered, amphigenous, often on all four faces of a single needle, occasionally episquamous. Aeciospores 30–47(–55) × 21–34μ; wall, excluding warts, 0.3–1.5μ, hyaline; warts truncate, more or less stellate, finer than in *C. ledicola*, 1.0–2.3μ high × 0.3–1.5μ wide × 1.0–2.6μ spacing. Out of 11 specimens examined only two, Rel. Farl. 954 from Mt. Desert I., Me., and a collection from Lunenburg Co., N.S., yielded spores up to 55μ long; in the remainder the maximum was 47μ or less. As in *C. ledicola*, there is a suggestion that the spores are smallest in far northern collections. Such variation occurs between collections that aeciospore size is of no aid in separating *C. Empetri* and *C. ledicola*. Uredinia scattered, epiphyllous. Urediniospores 25–49 × 20–31.5μ; wall, excluding warts, 0.2–1.0μ, hyaline; warts cylindrical to slightly stellate or irregular, 0.7–2.2μ high × 0.3–1.0μ wide × 0.7–2.5(–3.0)μ spacing. Comparison with the figures for *C. ledicola* shows that the two species are distinguishable only by wall thickness and size of warts.

Telia were not seen at Great Whale River. They may have been largely past when the writer arrived in the third week of June since growth of spruce had started, but were probably simply overlooked owing to the pressure of other activities. Subsequent events proved that they must have been at least locally abundant. Faull (5) describes them as epiphyllous, but on the recurved leaf margins, yellow, cushion-shaped, rupturing the overlying epidermis at maturity; teliospores 19–24 × 18–21μ, thin-walled, three to six in a chain, germinating at maturity in early summer.

Specimens examined give the following range extensions: *Picea Engelmanni*, B.C. *P. glauca*, N. Que., N.S. *P. rubens*, Me. *P. sitchensis*, Alaska. *Empetrum nigrum*, Alaska, Yukon, Mack., Frank., Man., N. Que., N.S. Faull (l.c.) found the aecia on *P. glauca* in eastern Quebec and secured them by culture on *P. glauca* and *P. rubens*. He also recorded the rust on *E atropurpureum*, Que.; *E. Eamesii*, Nfld.; and *E. rubrum*, Falkland Is.

Chrysomyxa roanensis (Arth.) Arth.

This species is known only from Tennessee on *Rhododendron* spp. A *Peridermium* on *Picea rubens*, collected 24 Sept. 1935 on Mt. LeConte (type locality of *C. roanensis*), Great Smoky Mts. National Park, Tenn., by H.M. Jennison (Herb. Arth. 47883), almost certainly belongs to this species. Pycnia and aecia amphigenous. Aeciospores 27–45(–62) × 19–26.5μ; wall, excluding warts, less than 0.5μ, hyaline; warts more or less cylindrical 1.0–2.0μ high × 0.4–1.3μ wide × 1.0–2.5μ spacing. The uredinia are scattered and hypophyllous. A specimen of the uredinial stage on *R. punctatum*, from Chimneys, Tenn., (Herb. Arth. 44395) has spores 28–42(–47) × 19–25.5μ; wall less than 0.5μ; warts more or less cylindrical, 1.3–2.8μ high × 0.3–1.3μ wide × 0.8–2.5μ spacing. The close correlation in size and shape of spores, thickness and ornamentation of walls, and locality leaves little doubt of the connection between these specimens. As discussed below, *C. ilicina*, the only other species likely to occur in the area, probably has very different aeciospores. The moderately elongate spores of *C. roanensis* suggest that it is related to *C. ledicola* and *C. Empetri* on the one hand and to *C. Piperiana* on the other. Occasional spores are quite narrowly elongate.

Chrysomyxa Piperiana (Arth.) Sacc. & Trott.

Chrysomyxa Piperiana has not been recorded from Canada, but occurs on the Pacific coast in California, Oregon, and Washington on *Rhododendron californicum*. It may possibly occur in the extreme south of British Columbia, but it is probably limited in its northward extension by the slow development of the aecia, apparently an adaptation to a Mediterranean type of climate. Aecia at Trinidad, Calif., mature between September and December. *Peridermium Parksianum* Faull, on *Picea sitchensis*, as indicated by spore correlation, the field observations of H. E. Parks and cultures by Faull (4), is the aecial stage. In Calif. Fungi 426, the type collection, the aeciospores were found to be 40–126 × 12.5–21(–24)μ, the longer spores taper-pointed, the shorter often truncate; wall usually longitudinally angled, thin, apparently 0.5μ or less, hyaline; warts slender, cylindrical to more or less stellate, 0.5–2.0μ high × 0.2–0.8μ wide × 0.6–1.7μ spacing. Herb. Arth. 47293 from the same locality yielded spores 47–120 × 10–17μ; wall less than 0.5μ, hyaline; warts 0.9–1.8μ high × 0.3–0.9μ wide × 0.7–2.0μ spacing. In both these collections, the pycnia and aecia are strictly epiphyllous in so far as can be determined without sectioning all leaves, not hypophyllous as stated by Faull (3). *P. sitchensis* shows rather more distinction between the upper and lower leaf surfaces than do some other species. If the aecia are ever found on *P. glauca* or *P. Engelmanni*, they may prove to be amphigenous. Although several mounts were made no spores less than 40μ long could be found. If spores as short as the 25μ minimum given by Faull occur, they are rare. The frequent occurrence of broken spores, which may simulate short, truncate ones, makes measurement under the oil-immersion objective advisable even with such large spores. The uredinia are scattered and hypophyllous. A specimen from Del Norte Co., Calif. (Herb. Arth. 44389) yielded spores

(36–)44–77 × 15.5–24μ, occasionally longitudinally ridged; wall about 0.5μ, hyaline; warts more or less cylindrical, 1.0–2.2μ high × 0.3–1.0μ wide × 1.0–2.0μ spacing. The correlation between spore forms is remarkably close in all characters except maximum length.

Chrysomyxa Ledi (Alb. & Schw.) de Bary

After several attempts to find points of distinction between the aecial stages of *Chrysomyxa Ledi* and *C. Cassandrae*, the writer made detailed measurements from the culture collections of the late Prof. W. P. Fraser. Absolutely no safe distinction could be found in any character of the aeciospores or the peridial cells. Study of a number of uredinial collections showed that the supposed distinctions in this stage were largely unreal, the spores being just separable by the form of their wall markings. The recent collection of *C. Rhododendri* in the District of Mackenzie led to the discovery that its uredinia are also almost indistinguishable from those of *C. Ledi*; and study of European material of the aecial stage showed no convincing distinction. It seems advisable to treat these three rusts as varieties of a single species, if only to discourage attempts at separation of the Peridermia except when exclusively associated with a single alternate host.

Chrysomyxa Ledi (Alb. & Schw.) de Bary var. **Ledi** n. n.

The following description of the aecial stage is based on several mounts from Fraser's cultures of 1910 and 1911 on *Picea rubens* in Nova Scotia: Aeciospores 18–32 × 13.5–25μ; wall, including warts, 1.5–3.3μ; warts narrowly cylindrical to slightly irregular, at 1.0–2.3μ spacing. The uredinia are scattered and hypophyllous. On the basis of several widely separated collections, the urediniospores were found to be 19–34.5 × 12.5–20.5μ; wall, including warts, 1.0–2.0μ, hyaline; warts shallow, flattened, polygonal to irregularly elongate, too closely spaced to allow accurate measurement of height, 0.5–2.5μ wide, occasionally to 7μ long.

Specimens seen give the following range extensions: *Picea mariana*, N.S. (*fide* W. P. Fraser, but no accompanying data). *Ledum glandulosum*, B.C. *L. groenlandicum*, Mack., Que. This rust does not appear to have been collected on *L. palustre* var. *decumbens*, partly, no doubt, because it is somewhat more southerly in range than *C. ledicola*, but it attacks *L. palustre* in Europe.

Chrysomyxa Ledi (Alb. & Schw.) de Bary var. **Cassandrae** (Peck & Clinton) n. comb.

The following description of the aecial stage is based on Fraser's cultures on *Picea glauca* and *P. rubens* in Nova Scotia: Aeciospores 18–30.5 × 13.5–24.5μ; wall, including warts, 1.5–3.0μ, hyaline; warts narrowly cylindrical, at 1.0–2.0μ spacing. Uredinia scattered, hypophyllous. Urediniospores, on basis of widely separated collections, 19–33.5 × 13.5–22μ; wall, including warts, 1.0–2.3μ, hyaline; warts more or less cylindrical to irregularly elongate, 0.3–1.0μ wide, occasionally to 2.5μ long.

Range extensions from specimens examined are: *Picea glauca*, N.S. *P. rubens*, N.S. *Chamaedaphne calyculata*, Yukon, Mack., Que.

Chrysomyxa Ledi (Alb. & Schw.) de Bary var. **Rhododendri** (DC.) n. comb.

The uredinial stage of this rust, previously unrecorded in North America, was collected on *Rhododendron lapponicum* at Indin L. (67° 17′ N, 115° 12′ W), District of Mackenzie, by W. J. Cody and B. J. McCanse, in August, 1949. Its distribution in Canada is not known, but it is evidently uncommon, for the writer has scrutinized many extensive collections of *R. lapponicum* without finding it and also examined numerous colonies at Great Whale River without success. The following description of the aecial stage is based on D. Sacc. Myc. Ital. 1645 bis and 1645B and Syd. Myc. Germ. 1161: Aecia scattered on leaves of *Picea excelsa* in Europe, not known in North America. Aeciospores 20–33 × 14–21μ; wall, including warts, 1.5–3.3μ, hyaline; warts narrowly cylindrical, at 1.0–1.8μ spacing. The spores have been described as up to 45μ long, but it is significant that Klebahn (6) gives "Sporen ellipsoidisch, 17–45 × 12–22μ (eig. Mess. 22–30 × 14–21μ)". It is unbelievable that this rust with small urediniospores should produce such long aeciospores. The explanation evidently is that peridial cells, some of which may be over 40μ long, more or less ellipsoidal and with warts only slightly coarser than those of the spores, have been taken for spores. The uredinia are scattered and hypophyllous. The spores, based on D. Sacc. Myc. Ital. 458 on *R. ferrugineum* and Linh. F. Hung. 243 and 246 on *R. myrthifolium* are 20–29.5(–34) × 14.5–22μ; wall, including warts, 1.2–2.0μ, hyaline; warts cylindrical to hemispherical, 0.3–1.0μ wide. In the collection on *R. lapponicum*, the spores are 19–29 × 15–20μ, with walls and markings indistinguishable from the other specimens. Curiously enough this rust does not seem to have been recorded on *R. lapponicum* in northern Europe.

The only appreciable distinctions between these three forms lie in the urediniospore warts. This distinction is reflected in a tendency for the warts on the aeciospores of *C. Ledi* var. *Ledi* to be slightly irregular. Other distinctions that have been put forward seem to be largely imaginary. Arthur (1) described the peridia of *C. Cassandrae* as firm, with cells abutted or slightly overlapping, and those of *C. Ledi* as delicate, with cells abutted (not overlapping); but examination of authentic material shows these characters to be inconstant. Telia are seldom of much taxonomic value in *Chrysomyxa* and this complex is no exception. In a single specimen of *C. Ledi* var. *Cassandrae* (DAOM 23441) from Cochrane, Ont., the teliospores are 9–24 × 8–17μ, covering the range given by Arthur for *C. Ledi* and obliterating any supposed distinctions. The telia of the *Rhododendron* rust have not been seen, but, from Klebahn's (6) figures of 20–30 × 10–14μ for the spores, it is safe to assume that they are not widely different. The many resemblances between these three forms, and their meager distinctions, make it undesirable to mask their relationship under separate specific names.

Specimens of unrelated peridermia give the following range extensions for the species *sensu lato*: *Picea Engelmanni*, Alta. (Vesterg. Micr. Rar. Sel. 1257,

at least in part, issued as *Peridermium decolorans*, which is the aecial stage of *C. ledicola*). *P. glauca*, Alaska, Man., Ont., P.E.I. *P. mariana*, Man., Que., P.E.I. *P. pungens*, Ont. *P. sitchensis*, B.C.

Chrysomyxa ilicina (Ell. & Ev.) Arth.

This species, on *Ilex opaca*, was long recorded only from West Virginia, but is now known also from northeastern Tennessee. It is perhaps more closely related to *C. Ledi* than to any other North American species. The uredinia and telia are scattered and hypophyllous and the urediniospores are small. The range of spore sizes differs little from that for *C. Ledi*, but long, narrow spores are commoner than in that species. The most distinctive feature of the urediniospores is the lack of warts over from one-sixth to nearly one-half of the surface of virtually every spore. This condition is not to be confused with the remnants of the primary wall that may overlie the warts in slightly immature spores of any *Chrysomyxa*. In *C. ilicina*, the smooth areas can often be seen to be bordered by minute, shallow, and very closely set warts, which indicates that the condition is not merely a mechanical effect, but is a definite pattern laid down by the action of the cytoplasm in the immature spore before the secondary thickening is formed. The smooth area may be on the side or the end of the spore. The following description is based on a specimen from Fayette, W. Va., collected by L. W. Nuttall (Herb. Arth. 44344) and one from Shady Valley, Tenn., collected by A. J. Sharp (Herb. Arth. 44346): Urediniospores 19–30(–33) × 16–22μ; wall, excluding warts, 0.6–1.0μ hyaline; warts absent over part of wall, elsewhere slender, more or less cylindrical, 0.5–2.0μ high × 0.3–0.9μ wide × 1.0–2.0 μ spacing, except at border of smooth areas where sometimes very small and closely set.

The aecial stage of *C. ilicina* is still unknown. A *Peridermium* localized in spruce leaves, with small spores having fine warts and a smooth area on the wall, if collected in the appropriate region, would almost certainly be this species.

Chrysomyxa Pyrolae (DC.) Rostr.

Chrysomyxa Pyrolae is circumboreal and is one of the more abundant species in North America. It is systemic in both phases, although the systemic nature of the aecial stage in the cones of *Picea* has received little attention. A few partly infected cones have been seen, but even in these the mycelium does not appear to be truly localized. Generally a cone is completely infected or completely sound. The mode of infection does not appear to have been studied in detail, but the mycelium evidently spreads with the developing cone and prevents normal differentiation of the tissues in the axis; for infected cones can be cut through far more easily than healthy ones. Both field observations and urediniospore measurements suggest some degree of host specialization. Most of the urediniospores in collections on *Moneses* are narrower than average, with thin walls and small warts, but a collection from Great Whale River, Que., (DAOM 23442) shows both spore types and it accordingly seems inadvisable to segregate the form on this host. Similarly,

the rust on *Pyrola grandiflora* has spores that tend to be slightly larger than average, but the difference seems to be too small and inconstant to merit varietal distinction. Three aecial collections from British Columbia, DAOM 23433 and 23434 on *Picea glauca* var. *albertiana* and DAOM 23435 on *P. mariana*, show maximum spore lengths in excess of 40μ, compared with maxima of 33 to 37μ in specimens from Montana, Ontario, Maine, and Nova Scotia. These figures suggest geographic variation; but all the British Columbia specimens were collected in 1949, a season of great abundance for this rust, and it is conceivable that large spores tend to preponderate under certain conditions of growth. More collections, particularly with data on the telial host, are needed before final conclusions can be drawn. As is the rule with systemic rusts, the conditions for infection seem to be exacting in this species. This is presumably the explanation for the great abundance of the peridermial stage in British Columbia in 1949. Conditions for infection of *Moneses* and *Pyrola* may be equally exacting, but, since the fungus is perennial in these hosts, differences from year to year are not apparent. The following description is based on specimens from a number of hosts and a wide range of localities: Aecia systemic, but not perennial, in the cones of *Picea*, fruiting on the cone scales. Aeciospores 22–37(–46) × 17–35μ; wall, including warts, 2.0–4.7μ, hyaline; warts usually tabular, polygonal to elongate, usually too closely set for determination of height, 1.5–4.7μ wide × 2.0–8.0(10.0)μ long. Uredinia hypophyllous, systemic, and perennial on *Moneses* and *Pyrola*, on *P. virens* occasionally on scapes and on upper surface of petioles and midribs. Urediniospores 19–33(–36) × 13–24μ; wall, excluding warts, 0.4–1.0μ, hyaline; warts circular to elongate, entire, 0.5–1.8μ high × 0.5–2.0μ wide, to 4.0μ long, spacing very variable, about 1.0–3.2μ discounting elongate warts.

The following range extensions may be noted from specimens seen: *Picea glauca*, Ont., Que., N.S. *P. glauca* var. *albertiana*, B.C. *P. mariana*, B.C., N.S. *Moneses uniflora*, N. Man., N. Que. *Pyrola americana*, B.C., Sask., Man., Ont., Que. *P. asarifolia*, Man. *P. elliptica*, Man., Que., N.S. *P. grandiflora*, Yukon, Keew., Frank. *P. minor*, N. Que. *P. secunda*, B.C., Mack., Alta., Man., N. Que. *P. virens* Schweigg. (*P. chlorantha* auth.) Mack., Que.

Chrysomyxa Chiogenis Diet.

Chrysomyxa Chiogenis might be more widely recorded if the host, *Chiogenes hispidula*, were more conspicuous. The rust is more or less systemic; the mycelium does not invade an entire plant, but does apparently run through the stems for some distance, for usually several successive leaves are infected and uniformly covered with uredinia. The leaves are so small that both the occurrence of the rust and its systemic condition are easily overlooked. The urediniospores closely resemble those of *C. Pyrolae* in size and form, differing only in having thinner walls and fluted, rather than entire, warts. The aecial stage is known only from the cultures of Faull (4), who obtained infection on needles of *Picea glauca* and *P. mariana*. From the resemblance of the uredinia to those of *C. Pyrolae*, the writer would have expected the aecial habit to

be similar. It is still possible that the cones may be susceptible, but until further evidence is obtained it is best to assume that the species is only foliicolous. The writer has not seen the aecial stage and it is not possible to tell from Faull's description of the spores as 15–18 × 19–30μ, with walls closely and rather finely to subcoarsely warted, hyaline, and 2–3μ thick, how it may best be distinguished from *C. ledi*. It is probable that the warts are elongate and fluted. The following description of the uredinia is based on specimens from Michigan, Ontario, Quebec, and Nova Scotia, from all of which the species is unrecorded by Arthur (1): Uredinia hypophyllous, sparingly systemic. Urediniospores 17.5–34(–42) × 12–22μ; wall, excluding warts, less than 0.5μ, including warts 1.0–2.5μ, hyaline; warts stellate to fluted elongate, 0.7–2.0μ high × 0.5–1.3μ wide, to 4.5(–6.5)μ long, × 1.2–3.3μ spacing excluding elongate warts.

Chrysomyxa Woronini Tranz.

This remarkable rust, hitherto unrecorded in North America, was found at Great Whale River, Que., on *Picea glauca*, and west of Dawson, Yukon, on *Ledum palustre* var. *decumbens*, in 1949. It resembles *C. Pyrolae* in being systemic in *Picea* and systemic and perennial in its telial host, but is widely different in all other respects, notably the lack of uredinia. It is probably more or less circumboreal, for, in addition to its occurrence in northern Europe, it is reported by Tranzschel (10) from Kamchatka. It may sometimes be an important species near tree line, but probably never occurs far inside it, since the telia appear to be restricted to *Ledum palustre* and its new world variety. Tranzschel's (9) original description served mainly to distinguish this species from *C. Ledi*, with which some European writers have, nevertheless, continued to confuse it; he also indicated from constant association during two seasons' study that *Peridermium coruscans* Fr. was the aecial stage. Klebahn's failure (6, p. 712) to prove the connection by cultures with telia sent to him in Germany in no way invalidates Tranzschel's conclusions. The difficulty of securing infection with many systemic rusts is notorious, and the likelihood is remote of optimum conditions being found in Germany for this northern species. The details of the life cycle are not known, but, since the two telial collections examined were both germinating when collected in July, it appears that the mycelium from the basidiospores must invade the growing point of the spruce and overwinter in the terminal bud. Of the usual cluster of one apical and two lateral buds on the tip of a branch, from one to three may produce infected shoots. The mycelium develops with the new shoot, stunting it to less than an inch in length and producing such masses of spores from aecia crowded on the closely packed needles that the whole structure suggests a staminate strobilus. In Romell, F. Exs. Scand. 158, on *P. exscelsa*, from near Stockholm, spores were being shed on 31 May; but in the writer's two collections (DAOM 23443 and 23444) on *P. glauca*, from Great Whale River, spores were being shed in abundance on 13 and 18 July. The state of development of the trees is not very different and most of the apparent anomaly is probably due to climatic differences, the spring at Great Whale River being

delayed by the flow of the prevailing winds across ice-laden Hudson Bay and that of Stockholm being presumably advanced by the dynamic warming of maritime air passing over high land. It seems certain that new growth of *Ledum palustre* must always be under way before the aeciospores are shed. Infection is presumably through the growing points and the mycelium must overwinter in the terminal buds. Tranzschel points out that the telia, which almost completely cover the lower leaf surface, are borne on the new season's leaves only. The witches'-brooms that are formed indicate that the mycelium is perennial, but the infected leaves are evidently shed before the second growing season. The complete life cycle, from telia to telia, thus appears to require two years. The abundance of *Ledum palustre* var. *decumbens* at tree line throughout northern Canada suggests that *C. Woronini* may rank with *C. Empetri* and *C. ledicola*, where weather conditions favor it, as one of the complex of factors deterring the invasion of the barrens by spruce. The aecial collections from Great Whale River were from stations nearly a mile apart; one consisted of three shoots, and the other of a considerable number on a clump of small trees with many dead tips that seemed to be due partly to old infections and partly to infections that failed to develop aecia. The latter site was at the edge of an active sand dune, which may have buried the infected *Ledum* before the *Peridermium* was discovered; but the identity of the rust was then unknown and, in the absence of any urgent reason for examination of the *Ledum*, the small brooms may easily have been overlooked. The Yukon collection (DAOM 23445), by J. A. Calder and L. G. Billard, consisted of a single broom taken with a mass collection of *C. ledicola*.

A detailed description follows: Aecia systemic in the shoots of *Picea*, abundant and amphigenous on the crowded leaves of the greatly shortened axis, and producing great numbers of spores. Aeciospores (27–)33–62(–66) × (16–)19–34(–37)μ on *Picea glauca*, Quebec, 29.5–56(–62) × 19–36(–45)μ on *P. excelsa*, Sweden (Romell, F. Exs. Scand. 158); walls, excluding warts, 1.0–2.5μ, hyaline; warts truncate, polygonal, 1.2–2.5μ high × 0.6–2.0μ wide × 1.2–2.8μ spacing. Uredinia lacking. Telia, on basis of Yukon collection and Vesterg. Mic. Rar. Sel. 1004b, forming small witches'-brooms on *Ledum palustre* and *L. palustre* var. *decumbens*, hypophyllous and crowded on current season's leaves, in crusts about 50–90μ high at center. Teliospores 18.5–40 × 12–19μ, wall thin, hyaline. Basidia 8.5–10.5μ wide, abruptly curved above. Basidiospores 8.5–11.5 × 6.5–9.5μ, reniform to suborbicular. Basidia and basidiospores orange pigmented.

Chrysomyxa Weirii Jackson

Chrysomyxa Weirii, a microcyclic species with localized telia on the leaves of *Picea*, was claimed by Arthur (1) to be correlated with *C. Pyrolae* on the basis of teliospore width and geographic distribution, but the evidence is not convincing. *C. Weirii* is of limited distribution in North America, whereas *C. Pyrolae* is circumboreal. If the two species were correlated, the telia of *C. Weirii* should be systemic on spruce cones instead of scattered on the needles. Little need be added to the description given by Arthur save to

note that the spores run up to 31 rather than 28μ long. Additional records are: *Picea sitchensis*, B. C. *P.* sp., N.S. The latter, if a native tree, must have been *P. glauca*, but it may have been a cultivated species and the rust may have been introduced with it. It should be noted, however, that Crowell (2) reported its occurrence on *P. rubens* in New Brunswick, in 1938, one year before the Nova Scotia specimen was collected.

Chrysomyxa Arctostaphyli Diet.

Chrysomyxa Arctostaphyli is a microcyclic species with localized telia on *Arctostaphylos Uva-ursi*. Its affinities are unknown and it is remarkable in being an exception to Tranzschel's law, since it occurs on the telial rather than the aecial host of its presumptive long-cycled ancestor. Sydow and Sydow (8) list three Asiatic species of the same type, on *Rhododendron* and *Menziesia*; none of these have been seen and the writer is not prepared to say whether they are all morphologically distinct. The telia of *C. Arctostaphyli* are localized and crowded in hypophyllous groups. Teliospores 23–64 × 13–18μ; wall thin. Basidiospores 8.5–9.5 × 7.5–8.0μ, broadly ellipsoid to subspherical, papillate, with orange pigment. This species is abundant in Yukon and Mackenzie, but its known range has also been enlarged by collections from Manitoba, Ontario, and Quebec. It thus appears to be nearly coextensive with its host in North America, although it is commoner in the west than in the east.

Acknowledgments

The writer wishes to express his thanks to Dr. G. B. Cummins, Purdue University, for material of species that do not occur in Canada, which allowed the completion of morphological keys to the genus in North America. Thanks are also due to Mr. I. L. Conners, of this Division, whose assistance and encouragement greatly facilitated completion of this study, and to the various members of this Division who have enthusiastically collected rust specimens on the writer's behalf while engaged in field work across Canada.

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INCREASE IN GIRTH OF THE CAMBIUM IN *THUJA OCCIDENTALIS* L.[1]

By Barbara E. Whalley[2]

Abstract

Studies of serial tangential sections of the secondary xylem indicated that increase in girth of the cambium was accomplished not by regulated divisions spaced merely to bring about the required increase in circumference, but by such rapid multiplication of initials as to produce a great superfluity if all survived. Owing to the rapid production of new initials and the associated high ratio of disappearance, the pattern of cells in the cambium changed quickly. This necessitated extensive intercellular adjustments which apparently were of rapid completion. No support was found for Priestley's theory that cell adjustments in the cambium are gradual and of a "symplastic" nature.

Introduction

The problem of the increase in girth of the cambium which accompanies the production of secondary xylem has engaged the attention of anatomists for some time. Nägeli (see Bailey, 2) was one of the first to speculate on the mode of division by which new cambial cells were formed. He proposed a theoretical scheme in which there was a continued simple multiplication, cambial cells dividing by radiolongitudinal walls. Later work by Krabbe (Scott, 8), Bailey (2), Klinken (4) has demonstrated that while this type of division is to be found in angiosperms with stratified cambia, it does not occur in the conifers. Here the fusiform initials are very long and divide by pseudotransverse walls, the mother cell being divided into two daughter cells which lie one above the other. These daughter fusiform initials then elongate until they become as long as or longer than the original fusiform cell, growing past each other in the process of elongation.

The manner of cellular adjustments taking place in the cambium following the pseudotransverse divisions of the initials has long been the subject of controversy. Krabbe (Scott, 8) was the first to apply the term "sliding" growth to these adjustments. According to his view, each cell grew independently, growth probably being localized at the points where it penetrated between the neighboring cells. As a consequence of this growth the cells shifted in relation to each other, forming new cell contacts. Klinken (4) applied this concept to the cambium of conifers, basing his observations on serial tangential sections of the secondary phloem of *Taxus baccata* L. Priestley (6, 7) suggested an alternative hypothesis to the "sliding "growth theory which he called "symplastic" adjustment. By this he meant that the cells did not grow independently, slipping past one another, but all together as a unit. The primary walls grew at the same rate so that no slip was necessary. Sinnott and Bloch (9) made direct observations of the apical root meristem of certain

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grasses and applied the term differential wall growth to the type of adjustment found there. Different parts of the cell wall extend at different rates, one end of the cell being affected by a "wave" of elongation before the other. They applied the term "intrusive" growth to special cases previously described as "sliding" growth, such as the sharply localized growth of cambial cells. Meeuse (5) after making a comprehensive survey of the literature concerned with intercellular adjustments in the cambium came to the conclusion that neither "sliding" nor "intrusive" growth was concerned with the increase in girth of the cambium.

Priestley's hypothesis was a new and interesting approach to the problem and stimulated further investigation on the subject of cell adjustments generally, though not of those in the cambium itself. Priestley suggested that the mutual adjustments of cambial initials took place slowly, with only a small number of pseudotransverse divisions being required to produce the necessary numerical increase in fusiform initials. Unfortunately his theory was based on theoretical considerations rather than direct observation. If the various theories are to be assessed, it would seem necessary to acquire further information on the actual behavior of the cambial cells, particularly as regards the frequency of pseudotransverse divisions, rate of elongation, mortality, number of conversions into ray initials and other relevant phenomena.

Materials and Methods

For the study of the succession of anticlinal divisions of the fusiform initials in the cambium, coniferous material was utilized because of the relatively slight change in shape which the derivative cells undergo during maturation. Among the native conifers, *Thuja occidentalis* L. was best adapted for the purpose owing to its availability, the absence of resin ducts in the xylem, and the fact that the fusiform initials are shorter than in the other genera (Bailey, 2). This reduced length of the fusiform initials simplified the work involved in making tracings from the serial sections.

In previous studies of anticlinal divisions in the cambium (Barghoorn (3), Klinken (4)), the procedure has been to cut serial sections from the secondary phloem since the phloem cells undergo less change in shape during maturation than do the elements of the secondary xylem. However, as the production of secondary phloem is usually very much less than that of secondary xylem, the record of change in the cambium as interpreted from the successive phloem elements in each radial row tends to be incomplete, compared with that in the more rapidly produced and more extensive xylem. The gaps between the successive stages are greater in the phloem and sometimes evidences of division appear in the xylem without counterpart in the phloem, because the fusiform initials failed to produce phloem cells during the interval in question. Further, in older material particularly, it is possible to trace the history of change for greater distances through the secondary xylem than the secondary phloem because of abscission or distortion of some of the latter tissues. For these reasons the secondary xylem was used for sectioning.

As the fusiform initials vary in length in different parts of the tree, wood samples were taken from the roots where the initials are long and from the branches where the length is much reduced. The root wood was obtained from horizontal roots 2.5–3.0 cm. in diameter taken a few feet from the trunk in the top few inches of soil. The lower branches 2.5–3.5 cm. in diameter were cut from a medium sized tree. Moderately large blocks of wood were softened, examined for traces of wounding, and if found to be free from injury and straight grained, were sectioned. Serial tangential sections 22μ thick were cut from the branch blocks, and sections 30μ thick from the root blocks. These were supplemented by radial sections 15μ in thickness.

From the serial tangential sections, tracings were made by projection on semitransparent paper to a magnification of ×180 or ×137, and in the roots ×79. A tracing was made from approximately every 10th section. The rows of tracheids succeeding each of the rows initially selected were followed by direct observation of every section, and were colored in on each tracing, a different color being used for the successors of each of the initially selected rows. The assumption was made that the rows of tracheids replicated the cambium at the various stages of its development, and that when a row of tracheids was followed by two the fusiform initial had undergone an anticlinal division at that point. Similarly it was assumed that when a row of tracheids came to an end, as was frequently the case, the fusiform initial had disappeared from the cambium.

The Succession of Anticlinal Divisions in the Cambium

Young Branch

The history of the succession of pseudotransverse divisions of fusiform initials in the cambium is presented in diagrammatic fashion in Fig. 1. In this figure are charted the successive anticlinal divisions of five fusiform initials *a–e* which took place during the production of 8–9 mm., of secondary xylem, representing the growth of twenty-eight years. It will be noted that these five fusiform initials behaved quite differently as regards the frequency and time of their divisions. Judging from the length of the tracheids, fusiform initial *a* at the start of the series was slightly less than 1.3 mm. long. It very soon divided to form two daughter initials, each of which divided again. One of these, represented by the second line from the top in Fig. 1, divided yet again, the third successive anticlinal division in the series. After this division, one of the two initials so produced stopped cutting off tracheids when about 0.5 mm. long, and evidently underwent conversion into one or more ray initials, with a single ray eventually being established. The fourth anticlinal division soon followed with the new pseudotransverse wall in this instance appearing near one end so that the division was definitely excentric. The smaller initial produced in this fashion shortly disappeared. The larger initial gradually lengthened and underwent a maximum of six more successive anticlinal divisions. In another part of the series, indicated by the third line from the top, one of the two daughter initials resulting from an anticlinal division was

converted to two ray initials. It is noteworthy that after the daughter fusiform initials had increased to nine, in the whole series, a surge of anticlinal divisions occurred with many of the new-formed daughter initials disappearing.

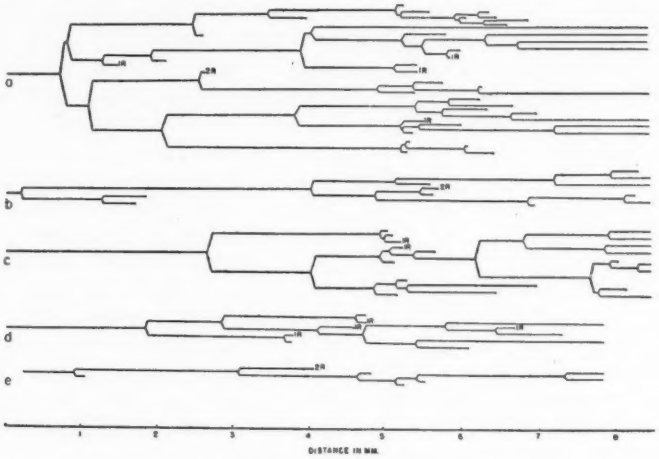


FIG. 1. *Schematic representation of the succession of tracheid rows belonging to five series a–c beginning near the pith of a branch. Termination of lines in small dots indicates cessation of the rows, and the origin of rays is indicated by the letter R with the digit showing the number of rays formed in each instance. The diagram may be interpreted as showing the time and frequency of anticlinal divisions of the fusiform initials, the point of disappearance of fusiform initials from the cambium, and the conversion of fusiform initials to ray initials.*

Eventually only eight fusiform initials survived. Over the period studied nine successive anticlinal divisions occurred with the various daughter initials undergoing a total of 38 pseudotransverse divisions. If all had survived, 39 rows of tracheids would have been formed, but 31 rows ceased at various times.

In the case of fusiform initial *c* there was moderate multiplication, a total of 18 divisions occurring, with 12 of the daughter initials disappearing and seven surviving. Fusiform initials *d* and *e* were the least successful of those studied. Initial *d* divided a total of nine times and initial *e* six times, but in each case only two of the daughter initials persisted.

Thus in the six lineal series shown in Fig. 1 there was a total of 71 anticlinal divisions during the production of 8–9 mm. of secondary xylem. Sixty-four initials vanished from the cambium and the net increase in number of functioning fusiform initials was only 23. That is, only one-third of the anticlinal divisions resulted in additions to the cambium.

The changes in the pattern of the cambium which follow as a consequence of the frequent multiplication and loss of fusiform initials are illustrated in Figs. 2 and 3, both these diagrams, which were made from the tracings, have of necessity been distorted, owing to the length of the tracheids.

At the outset the selected cells were contiguous and occupied a tangential distance of 0.06 mm. and a longitudinal distance of 2 mm. Eight mm. further outward, where the second diagram was made, the number of cells had

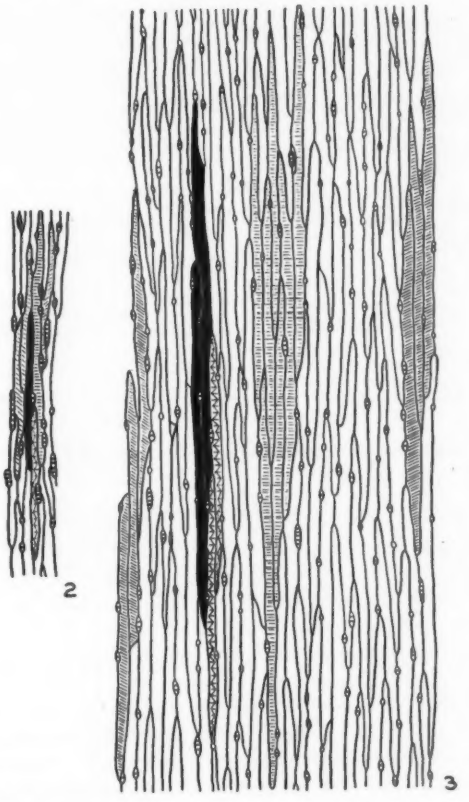


FIG. 2. *Diagram of tangential section near the pith showing the relative positions of the initial tracheids of the five series a–e, the history of which is illustrated in Fig. 1.*

FIG. 3. *Tangential section 9 mm. from pith showing the increase in number and dispersal of the tracheids belonging to the same five series.*

increased to 16, and these were spread over a tangential distance of 0.5 mm. and a longitudinal distance of 3.2 mm. The groups of derivative cells were no longer contiguous but had been separated by the inthrusting of unrelated cells.

The history of six lineal series was traced for 7 mm. in another young branch. In the six series 24 anticlinal divisions occurred but the net increase in number of fusiform initials was only eight. Altogether 16 fusiform initials disappeared from the cambium.

Old Branch

The succession of tracheid rows in the outer annual rings of an old branch 3.4 cm. in diameter is illustrated in Fig. 4. At the end of the 4 mm. distance

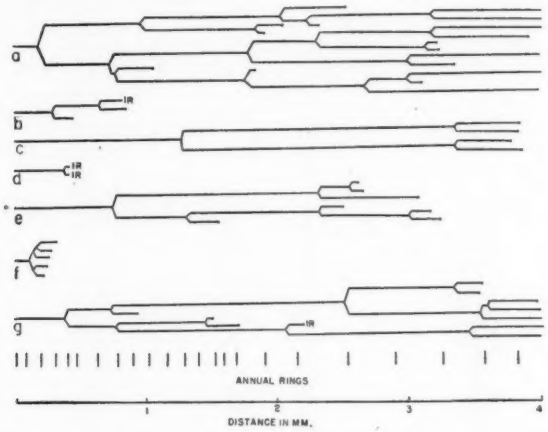


FIG. 4. *The succession of tracheid rows in seven series a–g in the outer annual rings of an old branch where there was little increase in girth.*

through which the rows were traced, there were but three more files of tracheids than at the beginning. The seven fusiform initials involved underwent repeated anticlinal divisions, but the net gain was only three, owing to the disappearance of 35 of the daughter initials.

Considering the individual histories briefly, it will be noted that fusiform initial *a* underwent the greatest number of pseudotransverse divisions. Eleven of the daughter initials disappeared so that after 16 anticlinal divisions only six initials persisted. Fusiform initial *g* divided anticlinally less frequently than did *a*, but again most of the daughter initials disappeared at different times and ultimately only four survived. The other initials *b–f* underwent anticlinal divisions several times but in all five series the daughter cells so produced vanished one after another until none was left.

The above observations were made from the outer annual rings where very little increase in circumference took place. It will be noted that here the rate of anticlinal division was not related to the increase in girth of the cambium. Theoretically only a few anticlinal divisions were required. However, divisions actually occurred with considerable frequency, but the gain in number of cambial cells was nearly equalled by the loss. As a consequence, the slight increase in number of cambial cells was accompanied by continued drastic alteration in cell pattern.

Root

Six lineal series beginning near the primary xylem of the root were traced through the secondary xylem for a radial distance of 4.8 mm. (Fig. 5). From

the pattern of the rows of tracheids it was clear that the fusiform initials in these six series underwent a total of 34 pseudotransverse divisions. Twenty-four fusiform initials survived and 16 disappeared or were converted to ray

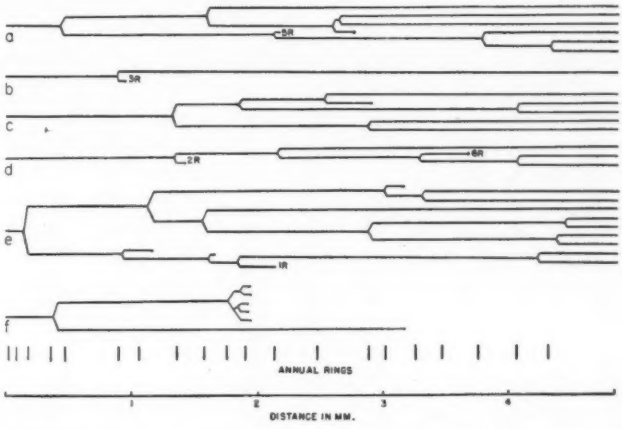


FIG. 5. *Diagrammatic representation of the sequence of tracheid rows in six series a–f beginning near the center of a root.*

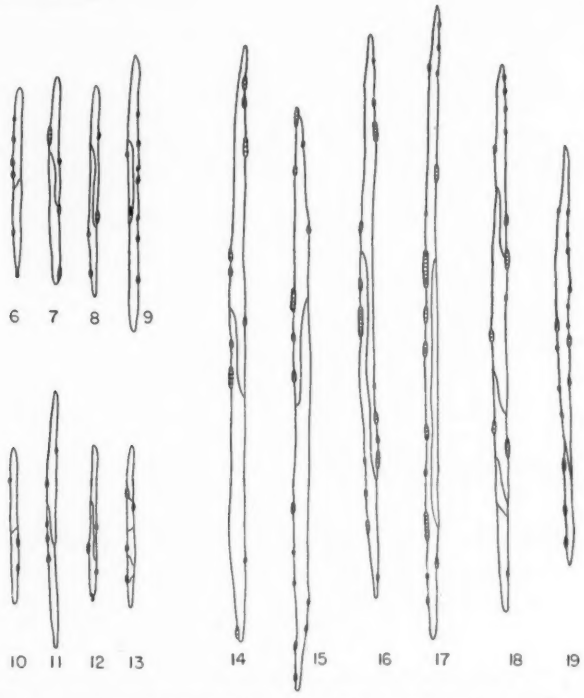
initials. The general history of change in the cambium of the root was thus similar to that observed in the branches. Frequent multiplication of fusiform initials by oblique anticlinal divisions took place, but many of the daughter initials vanished so that net increase was accomplished slowly.

The Nature of Anticlinal Divisions in the Cambium

Nearly all anticlinal divisions of fusiform initials were of the pseudotransverse type, as described by Klinken (4) and Bailey (2). Since the observations were made from the secondary xylem, after some elongation had taken place, it is not possible to make statements on the exact length of the anticlinal wall when first formed. However, there was much variation in the partition when first observed in the secondary xylem. Sometimes the pseudotransverse wall was almost horizontal (Figs. 6 and 10), duplicating the condition figured by Klinken (4); more often it was considerably longer (Figs. 7 and 11); and occasionally it was nearly one-third the length of the mother cell (Figs. 8 and 12). In the root the fusiform initials were considerably longer, a circumstance which might be expected to have some influence on the dividing wall. The latter ranged from short (Fig. 14) to very long (Fig. 16), the length on the whole being greater than in other parts of the tree.

Occasionally the first pseudotransverse division was followed by others, the fusiform initial becoming segmented into a series of short cells (Figs. 13 and 18). In some cases these shorter units divided periclinally a few times before

disappearing, the short cambial cells probably undergoing maturation into the last cell in each radial row. In other cases the short cambial cells survived to become ray initials.



FIGS. 6 TO 19. *Position and orientation of the wall in the first xylem element produced after an anticlinal division of the fusiform initial in the outer annual ring of an old branch. The width of the cells is exaggerated.* FIGS. 10 TO 13. *Same in young branch.* FIGS. 14 TO 19. *Position and orientation of the wall after anticlinal divisions in the root.*

Rarely the division in the fusiform initial was by an elongate anticlinal wall which cut off a small cell from the side (Figs. 9 and 17). This type of division was observed only three times. In two cases the smaller of the two cambial cells divided periclinally a few times, then disappeared. In the third case, in a root, five rays followed the disappearance of the smaller cell. This type of ray origin has been described by Barghoorn (3) as of frequent occurrence in coniferous cambia. Such was not the case in *Thuja*. Here most new rays were derived from the subdivision and reduction of the whole or a large part of the fusiform initials.

The Loss of Fusiform Initials

When rows of tracheids were about to stop there was usually a gradual decrease in tangential width of the successive cells, until the last was very

thin. In some cases there was also a reduction in length, the last tracheid in the row frequently being shorter, narrower, and with thinner walls than its predecessors. In some instances it seemed probable that the fusiform initial differentiated into a xylem or phloem element, the last in the radial row of cells.

Many of the fusiform initials which were lost from the cambium underwent conversion to ray initials. Sometimes the whole fusiform initial was subdivided into a series of shorter units, of which some vanished and others persisted to become ray initials. In other instances the formation of ray initials followed a pseudotransverse division with one of the daughter fusiform initials producing tracheids and sieve elements, and the other undergoing subdivision to ray initials. Occasionally the pseudotransverse division was excentric (Fig. 19), with the smaller of the two daughter cambial cells giving rise to ray initials. Rarely were ray initials formed from the ends or sides of fusiform initials.

Discussion

In his argument against "sliding" growth, Priestley (6, 7) attempted to show that elongation of fusiform initials in the cambium proceeded slowly, allowing ample time for gradual mutual adjustments of a "symplastic" nature. In support of his theory he referred to data presented by Bailey (2) on the size and number of cambial cells in one-year-old and 60-year-old stems of *Pinus Strobus*. From these data Priestley calculated that not more than four successive pseudotransverse divisions during the growth of 60 years would suffice to produce the required number of new cambial cells. It followed that if successive pseudotransverse divisions occurred only at 15-year intervals, the daughter cambial cells arising from such divisions would have 15 years in which to elongate to full size before the next subdivision.

It is clear from the data presented in this article that Priestley grossly underestimated the frequency of pseudotransverse division, largely because he failed to make adequate allowance for the repeated disappearance of fusiform initials from the cambium. Actually, considerably more fusiform initials disappear than survive, and consequently many more pseudotransverse divisions are required to maintain and increase the cambium than suggested by Priestley. For instance, in Fig. 1 it will be noted that fusiform initial *a* underwent 7 to 10 successive pseudotransverse divisions. If all the daughter fusiform initials survived and redivided, from 128 to 1024 fusiform initials would have been present at the end of the period of division. In reality there were but eight.

Obviously the increase in girth of the cambium is not accomplished by an orderly and well regulated anticlinal division of fusiform initials so as to produce only the number of cells required for the increase in circumference. Rather the cambium is a region of frequent anticlinal division, daughter initials being originated at such a rate that astronomical numbers would result in a short time were it not for the fact that most disappear. Owing to the rapid generation of new initials and the high ratio of disappearance, the pattern of

cells in the cambium does not remain fixed, as assumed by Priestley, but undergoes rapid change. New contacts between initials are being formed continuously and the required adjustments are completed quickly.

Acknowledgment

The writer wishes to express her sincere thanks to Prof. M. W. Bannan for his advice and kindly assistance throughout the course of this work.

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THE ELONGATION OF FUSIFORM CAMBIAL CELLS IN *CHAMAECYPARIS*[1]

By M. W. Bannan[2] and Barbara E. Whalley[3]

Abstract

Studies of the secondary xylem and phloem indicated that after their origin in anticlinal division, sister fusiform initials usually elongated rapidly, particularly at their overlapping tips. After this first phase, rate and amount of extension varied, both along the overlap and at the opposite ends. Often elongation proceeded in a somewhat periodic fashion, being determined in part by inherent factors and in part by position and behavior of the adjoining cells. Sometimes elongating tips were temporarily stalled at blocking rays, while at other times very rapid extension occurred in association with loss of a neighboring initial from the cambium. Growth appeared to be apical, "intrusive" in the sense that the elongating tips thrust between other cells. No evidence was found to support the theory of simultaneous elongation of considerable portions of adjoining walls by "symplastic" growth.

Introduction

In conifers the multiplication and enlargement of fusiform initials in the expanding cambium involve extensive intercellular adjustments. New cambial cells are formed by pseudotransverse division, the daughter initials subsequently elongating past one another. However, with regard to the precise site of elongation and the nature of the adjustments associated with the increasing overlap there has been much controversy and various theories have been proposed. Krabbe (17) suggested that in meristematic areas generally each cell grew independently with the result that some shifted in relation to others. Mischke (12), Klinken (10), Neeff (13), Bailey (2), Beijer (5), and others applied this concept of "sliding" growth to the elongation of cambial cells and their derivatives. Another method of cell growth and adjustment was proposed by Priestley (15). He argued that "sliding" growth was unlikely because plasmodesms would be shorn. As an alternative he suggested that the adjoining walls of neighboring cells elongated together over a considerable portion of their length without slipping by "symplastic" growth. More recently Sinnott and Bloch (18) concluded from observations on apical root meristems that wall growth of enlarging cells was sharply localized, being confined to the point of intrusion between other cells so that no slip occurred along previously formed contact faces. While no studies were made of the cambium it was suggested that the fusiform initials elongated by growth at the tips only, and the term "intrusive" growth was proposed. A similar mode of elongation, termed "interpositional" growth, was later advanced by Schoh-Bodmer (16) for the extension of fibers and pollen tubes.

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Among the theories mentioned, that of "symplastic" growth particularly was based largely upon theoretical considerations. One of the basic assumptions, as Meeuse (11) has stated in his broad application of the theory, was that intercellular adjustments "come about very gradually". As supporting evidence Klinken's (10) observations on *Taxus* have been cited. From a limited study of a single piece of stem Klinken reported that successive anticlinal divisions of fusiform initials occurred at five-year intervals, a period considered sufficiently long by advocates of the "symplastic" theory to allow slow elongation of the daughter initials and gradual intercellular adjustments. Priestley (14) estimated that multiplication of cambial cells was even less frequent, having calculated from data on cell size supplied in an earlier paper by Bailey (1) that a pseudotransverse division every 15 years was sufficient to produce the required number of new initials.

In order to acquire information on the actual frequency of anticlinal divisions involved in the multiplication of cambial cells, investigations were carried out in this laboratory on *Thuja* and *Chamaecyparis*. The results of these studies have revealed that Priestley's estimate was much too low. Under some circumstances, as in the growth of vigorous young stems, fusiform initials undergo three or four successive pseudotransverse divisions within a single growing season, and even in old specimens where annual increase in girth is slight, multiplication of fusiform initials may continue at a surprising rate. Production of new initials is accompanied by elimination which sometimes proceeds almost as rapidly. As a consequence of the continuous multiplication and loss of fusiform initials the pattern of cells in the cambium does not long remain fixed, as assumed by supporters of the "symplastic" theory. Instead, it is constantly changing with extensive adjustments resulting, some of which are of rapid consummation. Data on the frequency of anticlinal division and the extent of loss of cambial cells are to be found in previous reports (Bannan (3) and Whalley (19)), while the present paper deals with the elongation of fusiform initials after their origin in anticlinal division.

Methods

Since both xylem and phloem replicate the cambium, either may be used for determination of past changes in cellular pattern of that meristem. However, for the reasons cited in an accompanying article (Whalley (19)), the xylem was selected in preference to the phloem. Serial tangential sections were cut, and, from these, measurements were made of the length of successive tracheids for the purpose of obtaining information on the elongation of the initiating cambial cells. Of course it must be recognized that information so acquired is only approximate. Allowance has to be made for limited elongation during differentiation of the tracheids, the extent of which seems to vary from one cell to another. Cognizance must also be taken of the fact that in fast-growing specimens periclinal divisions may occur in a zone more than one cell wide. Hence some of the irregularities in length of successive tracheids may be due not to briefly intermittent elongation of the producing cambial cell, but rather

to different amounts of elongation on the part of the initiating cambial cell and its periclinally dividing derivatives.

Elongation of the Overlapping Tips of Sister Fusiform Initials

Multiplication of fusiform cambial cells in the conifers is effected by pseudotransverse division, an oblique anticlinal wall usually being formed near the center of the initial undergoing subdivision. The length of this wall was found to vary considerably. Observations on the derived cells in the secondary xylem indicated a range from short and almost transverse (Fig. 1) to obliquely longitudinal with a length up to one-third that of the mother cell (Fig. 2). Here again the limitations of the method are apparent, for the precise length of the wall in the dividing cambial cell could not be determined from either xylem or phloem since some elongation had already taken place before the production of the first derivative elements. Some idea of the extent of this early elongation may be obtained from a series of comparative measurements made by the authors on the length of the corresponding anticlinal partitions when first observed in sister cells of the xylem and phloem of *Thuja occidentalis*. Determinations from more than a hundred divisions in several stem and branch specimens revealed the anticlinal wall to be longer in the xylem in 88% of the cases. In only 7% was it longer in the phloem. Fluctuation occurred from one division to another and substantial degrees of difference were observed in various specimens, but on the average the dividing wall was 27% longer in the xylem than in the phloem. At the same time the combined vertical extent of the derivatives of the two sister cambial cells was only 5% greater in the xylem. Greater amount of overlap in the xylem may have been due in part to elongation during differentiation, but it is not to be expected that this exceeded 5%. Rather it would appear that the first periclinal division after subdivision of the fusiform initial was generally toward the phloem, with considerable elongation of the two newly formed sister initials occurring before the next periclinal division, apparently toward the xylem, took place. Presumably the amount of this early elongation was not less than 21% of the first observed overlap in the xylem.

Stages in the progress of elongation of the two sister initials originating in pseudotransverse division were followed in *Chamaecyparis*. Nearly a hundred cases were selected in which both sister initials survived and increased in size until one or the other was in turn subdivided. Measurements were made of the length of the anticlinal wall and of the total length of the overlapping tracheids, both immediately after pseudotransverse division and through the successive stages of elongation of the sister fusiform initials. Length of the anticlinal wall when first observed varied from one instance to another and in different parts of the tree, but the average in the nearly hundred cases selected was 431μ. If the early growth of the two sister initials proceeded at the same rate as in *Thuja*, it is to be assumed that the anticlinal walls had already undergone an average extension of at least 90μ at the time the first tracheid was produced. In this connection it might be noted that the term

anticlinal wall is used to denote a 3-ply system made up of two primary walls and the middle lamella according to the terminology proposed by Kerr and Bailey (9). The extension of this wall is due to the independent growth in opposite directions of the overlying tips of the two sister initials. For brevity the total growth of the overlapping ends is sometimes referred to simply as the increase in amount of overlap, or the lengthening of the anticlinal wall.

The approximate amount of further elongation was determined from serial tangential sections of the xylem 22 or 30μ thick. The average extension of the anticlinal wall between the section in which it was first observed and the following section was 85μ, and must have been due for the most part to the combined elongation of the overlapping tips of the two sister initials. This lengthening occurred during the production of one-two early-wood or two-four late-wood tracheids, but the actual number of times that the initiating cambial cells divided periclinally is, of course, unknown. In some cases there was probably local multiplication of elements by periclinal division of recently derived cells on the xylem side of the cambium. Elongation between the second and third sections was less than between the first and second, averaging 46μ. Much variation was noted in different cases. Extension between the first and second sections ranged from 460μ to slight shortening, the latter possibly being a maturation phenomenon. Between the second and third sections the range was from 240μ to a small negative value. Fig. 3 illustrates the most rapid elongation noted. In this instance the anticlinal wall increased from 130μ in one section to 590μ in the next. It is significant that extension of the overlap of the two sister initials greatly exceeded elongation at the opposite ends. Growth obviously did not conform to Priestley's scheme, for if such were the case the distance between the extreme tips should have increased by not less than the growth in the region of overlap. It is also significant that one of the overlapping ends grew more than the other.

In some cases the anticlinal wall did not undergo much further extension, while on other occasions rapid growth followed until the overlap shortly became two-three or rarely as much as five times the first observed extent. Two examples will be cited to show the range of behavior. In the first instance the anticlinal wall when first seen in the xylem was 640μ long; through the next eight serial tangential sections, each 30μ thick, it remained 700μ long; and 12 sections later it had increased only another 100μ to 800μ. In the second instance the anticlinal wall extended from 120 to 225μ during the

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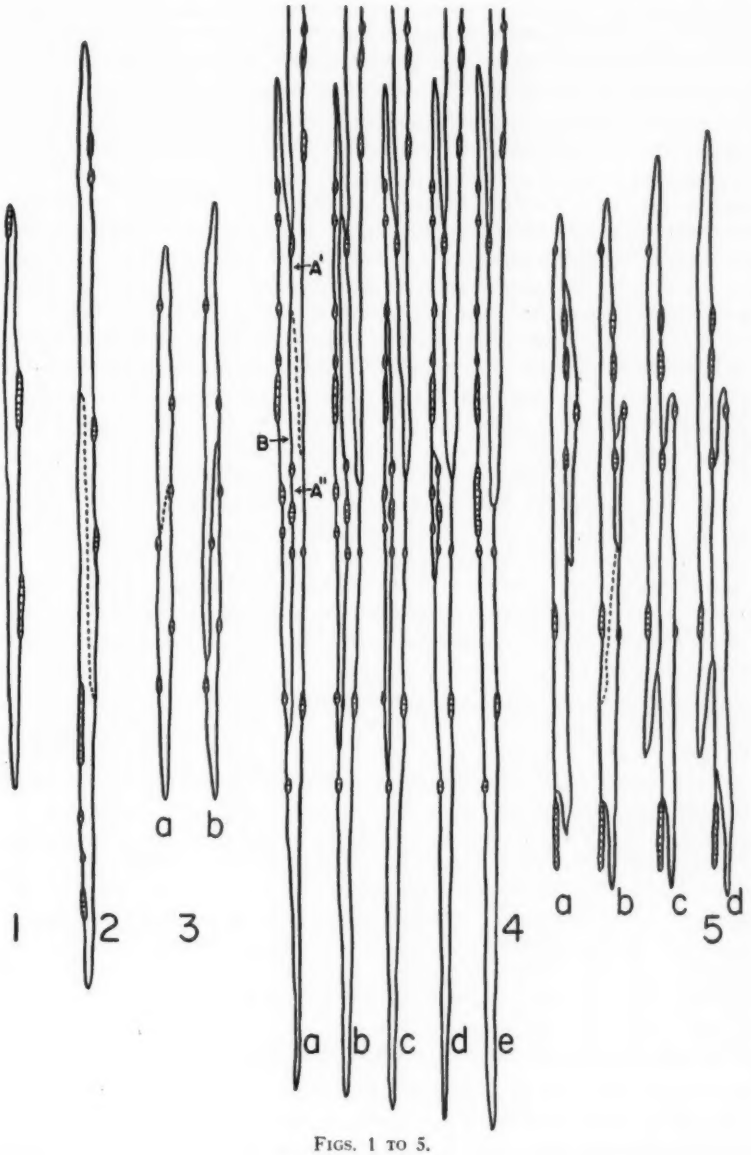
FIGS. 1–5. *Chamaecyparis thyoides. Diagrams from tangential sections of the secondary xylem which are to be interpreted as showing the nature of the anticlinal divisions involved in the multiplication of fusiform initials, and the subsequent changes in length of the sister fusiform initials. In all cases the width of the cells has been slightly exaggerated.* FIGS. 1 AND 2. *Range in length of the anticlinal wall when first observed in the derived xylem elements. Outer wood of old stem.* FIG. 3. *Rapid increase in overlap of tracheids derived from two sister initials, as seen in successive sections 22μ thick. Branch.* FIG. 4. *Increase in overlap associated with loss from the cambium of an adjoining fusiform initial. The stages illustrated took place during the production of 400μ of secondary xylem, approximately one-half the width of the annual ring. Old stem.* FIG. 5. *Rapid increase in overlap following loss of neighboring cambial cell, as seen in successive sections 22μ thick. Young stem.*



FIGS. 1 TO 5.

radial growth of 30μ included in the first section. By the end of the second it had reached 380μ, and three sections later was 625μ in length. It should not be concluded from these examples that the shortest dividing walls always underwent the greatest elongation for such was not necessarily the case. There was much variation in the initial length and in subsequent behavior.

Where the overlap became extensive, growth sometimes continued at a more or less uniform rate, but more often it proceeded in a fitful manner. Generally the rate and amount of elongation appeared to be influenced by the situation and behavior of the adjoining cells. Loss of cambial cells was of frequent occurrence, and when the tips of fusiform initials were favorably located they tended to elongate very rapidly into the "space" so provided. Fig. 4 illustrates the stages in such development. In Fig. 4*a* the overlap between the derivatives of two sister initials A′ and A″ was 610μ. Two sections later, as shown in Fig. 4*b*, the adjoining tracheid B became thinner, foreshadowing loss from the cambium of the producing fusiform initial, and the upper end of A″ began to thrust upward. In the next section, Fig. 4*c* the tracheid in row B was appreciably shorter at the upper end than its predecessor in Fig. 4*b*, and the upper tip of A″ had extended into the vacated area. Here there was an extension of 435μ in successive xylem elements derived from A″. After this sudden growth elongation proceeded slowly. In Fig. 4*e*, eight sections and 240μ after Fig. 4*d*, the file of shortening tracheids B came to an end, the rays on either side coalesced, and the overlap between A′ and A″ was 830μ greater than in Fig. 4*a*. Similar development is illustrated in Fig. 5. After an anticlinal division (Fig. 5*b*), the lower initial thrust upward into a vacated "space" so rapidly as to double the amount of overlap during the production of successive xylem elements.

The observations recorded above reveal that the overlapping ends of sister fusiform initials usually underwent early rapid elongation. Only rarely did they remain more or less stationary for a time. Subsequent behavior varied. Sometimes elongation proceeded in a steady, continuous fashion, whereas in other cases growth was erratic, extension apparently being influenced to a considerable extent by surrounding contacts. When a neighboring fusiform initial was lost from the cambium one or the other of the overlapping tips usually elongated rapidly into the "space" provided. After such a spurt growth generally reverted to a slower pace, until another opportunity was afforded for rapid extension. Obviously the sequence of events did not conform to the pattern of very gradual adjustments in a relatively static cambium such as has been assumed by supporters of the "symplastic" theory.

Elongation at the Opposite Ends of Sister Fusiform Initials

As was the case with growth of the overlapping ends, the rate and amount of elongation at the opposite tips of sister fusiform initials varied widely both among neighboring initials and from one specimen to another. On the whole the rate of extension was greater in young specimens than in old. Determinations from serial tangential sections of the xylem indicated the average

combined elongation at the two opposite ends of the sister initials to range from 0.70 to 1.25 mm. during the production of 1 mm. of secondary xylem in young stems and branches, and to vary from 0.36 to 1.19 mm. in old specimens. In these particular cases a radial distance of 1 mm. represented from 0.58 to 3.85 annual rings in young trees and 0.36 to 11.1 rings in the old specimens. The measurements relate to the increase in length of successive tracheids, but it may be assumed that elongation of the fusiform initials involved was only slightly less.

Sometimes elongation proceeded at a more or less uniform rate through spring and summer of successive years' growth, while in other cases elongation was more extensive during the summer. More often lengthening progressed in an erratic manner, depending to a large extent on adjoining cell contacts. For instance, elongating tips frequently became stalled at rays, as at the upper end in Fig. 6. Sometimes a considerable delay followed before the tip was able to thrust past the obstructing ray. In his investigation on

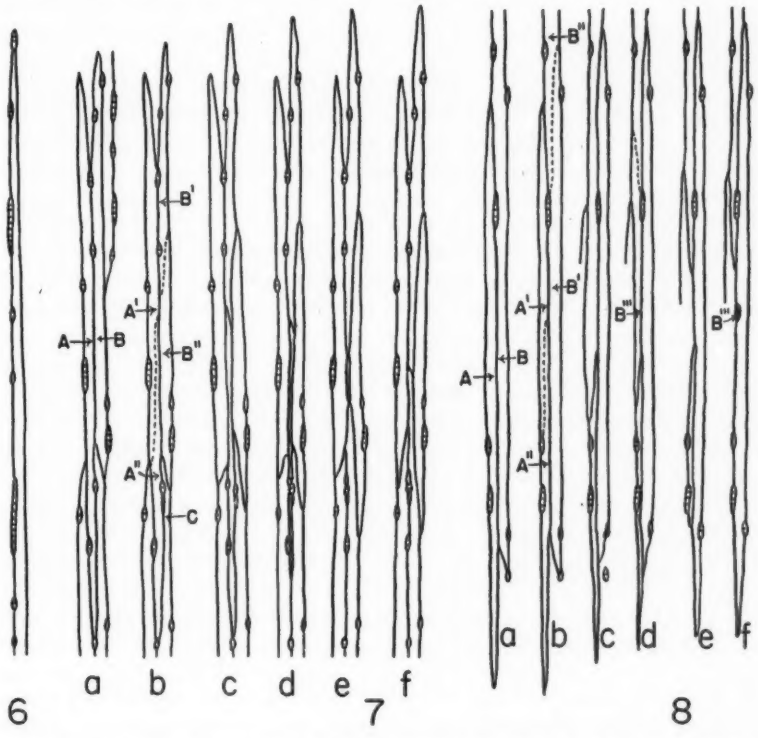


FIG. 6. *Chamaecyparis thyoides, old stem. Upward elongation of cell temporarily stalled at obstructing ray.* FIG. 7. *C. nootkatensis, old branch. Stages in elongation of tips of cells into "space" following loss of neighboring cambial initial.* FIG. 8. *C. thyoides, young branch. As above.*

the shape of fusiform initials Dodd (6) found some cells with truncate ends, and in view of Barghoorn's statements (4) on ray origin, interpreted these as having resulted from a cutting off of the tips of fusiform initials by transverse walls. The more probable interpretation of truncate ends is that they arise from contact, sometimes of lengthy duration, between elongating tips and obstructing rays of much earlier origin. Occasionally there was also a flattening, for a brief period, when the tips of two elongating fusiform initials (or their derived cells) first met, as at the point of contact of E and F in Fig. 9*b*. Infrequently certain tips lost their capacity for extension when there were no impeding rays or other obstructions, elongation having ceased for no obvious reason.

On the other hand, elongation was sometimes exceedingly rapid. This was especially true in the vicinity of disappearing fusiform initials. Examples of rapid extension of tips into "spaces" so provided are shown in Figs. 7 and 8. In Fig. 7 fusiform initials A and B divided anticlinally to produce daughter initials A′, A″, and B′, B″. Through succeeding periclinal divisions A″ decreased in size and simultaneously the tips of C and B′ elongated into the "space" so created, soon meeting (Fig. 7*f*). Even more rapid extension is depicted in Fig. 8. Here fusiform initials A and B were subdivided to produce daughter initials A′, A″, and B′, B″ (Fig. 8*b*). Immediately after these anticlinal divisions, in the next serial tangential section (Fig. 8*c*), the tracheid derived from A″ was appreciably smaller. No derivative of A′ was to be seen, the initial evidently having been lost from the cambium, and the tracheid derived from B″ extended downward into the "space" left by A′. Here the extension of B″, or its derived tracheid, amounted to 675μ from one section to the next, that is during the production of 22μ of secondary xylem or the equivalent of the width of one tracheid. This measurement was, of course, made from the xylem, but since the elongation was maintained in the following tracheid it seems logical to assume that the actual extension of the fusiform initial was almost as great. Shortly after this astonishing elongation, the initial B″ divided anticlinally forming B‴ which underwent conversion to a ray initial (Fig. 8*f*). With the reduction of B‴, the lower tip of B″ extended slightly downward.

As mentioned earlier, the rate and amount of growth varied widely among neighboring cambial cells and from one specimen to another. While some initials enlarged others became smaller, sometimes very slowly, sometimes quickly. Considerable differences in the inherent capacity to expand were indicated, differences which extended even to recently formed sister initials. The expression of these internal factors, however, was modified to a marked degree by the surrounding cell contacts, such as the presence of blocking rays or the providing of more space by the loss of a neighboring initial. In this connection reference should perhaps be made to the aspect of tracheid tips in strictly radial sections, particularly of old stem wood. If one were to judge from the appearance in some sections he might conclude that fusiform initials elongated only slightly if at all. Actually such deduction would be

in error. There is often very rapid elongation, but owing to greater confusion in the arrangement of the tips and to the fact that some may pass off the section because of growth to the side associated with loss of an adjoining cambial cell, the instances of rapid extension are not so obvious as those of little or no growth.

Comparative studies of the amount of elongation at the opposite (distal) tips and in the region of overlap of two sister fusiform initials yielded the following results. Immediately after their origin in anticlinal division the sister initials generally elongated more rapidly in the region of overlap than at the far tips, the reverse occurring only rarely. After this first phase, development followed diverse courses, but in many cases combined elongation of the opposite ends soon equalled that in the overlap. Ultimate amount of growth varied widely. In 42% of the cases studied total extension at the opposite ends surpassed that in the region of overlap, whereas in 51% the reverse was true. In 63% the differences in amount of elongation between the opposite tips and along the overlap exceeded 30%. Comparison of elongation of one of the distal tips with the other, that is, the opposite ends of the two sister initials, revealed some considerable differences. A difference exceeding 20% occurred in 74% of the cases. A few of the more noteworthy differences after varying periods of growth were 575μ vs. 0, 870μ vs. 50μ, 610μ vs. 70μ, and 1160μ vs. 120μ.

Occasionally substantial differences in elongation at opposite ends of the *same* fusiform initial were noted. Sometimes the explanation of the differences was not obvious, but in many cases they were clearly attributable to the influence of the local environment. For instance, one tip might become stalled at a blocking ray while the opposite end was undergoing rapid elongation into a "space" provided by loss of a neighboring fusiform initial.

Manner of Elongation of Fusiform Initials

Among the theories on the mode of elongation of fusiform initials, that of "symplastic" growth originated by Priestley (15) is the least acceptable. This theory was based on the following premises: the pattern of cells in the cambium alters only slightly over long periods of time, the production of new initials by pseudotransverse division is infrequent, the elongation of the daughter initials proceeds slowly, and the adjoining walls cannot elongate independently because plasmodesms would be shorn and the pits on the derived cells would not match. In fact, none of these assumptions is justified.

It has been shown in previous reports, Bannan (3) and Whalley (19), that the cellular pattern of the cambium is constantly changing as a consequence of the continuous production and elimination of fusiform initials. The accompanying intercellular adjustments are necessarily extensive and often are performed very rapidly. Further, the elongation of fusiform initials apparently proceeds by terminal extension, the growth of each cell occurring independently of its neighbor. No support for the hypothesis of simultaneous elongation of adjoining walls by "symplastic" growth is forthcoming from

study of the sequence of changes as revealed in the secondary xylem. A typical series of growth changes is illustrated in Fig. 9. Here the fusiform initial A underwent anticlinal division to produce two daughter initials

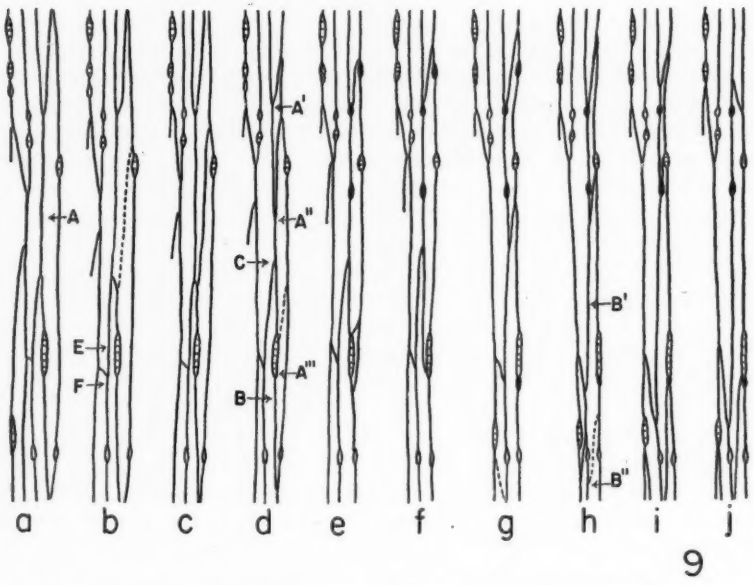


FIG. 9. *Chamaecyparis nootkatensis, old branch. Diagrams from tangential sections of the xylem showing successive stages in changing cell pattern. The changes illustrated occurred during the production of 300μ of secondary xylem, representing the growth of three years.*

(Fig. 9*b*), and shortly afterward the lower of these was in turn subdivided thus forming a vertical series of 3 initials, A′, A″, and A‴ (Fig. 9*d*). Almost immediately initial A′ was converted to three ray initials, the derived rays being shown in black (Fig. 9*e–j*), and A‴ was reduced to a single ray initial which became applied to the lower margin of an older adjoining ray (Fig. 9*g–j*). Meanwhile initial A″ elongated upward into the "space" left by reduction of A′ (Fig. 9*e*), after which shortening occurred from below upwards through successive periclinal divisions (Fig. 9*g–i*) until the initial disappeared from the cambium (Fig. 9*j*). With the progressive shortening of A″, B grew upwards, divided anticlinally to produce daughter initials B′ and B″ (Fig. 9*h*), and finally B′ occupied the "space" left by loss of A″. It is particularly worthy of note that while elongation of B and its daughter initial B′ was proceeding, its neighbor to the left, C, extended only very slightly. Thus the wall of B, and later B′, underwent considerable extension while the contacting wall of C remained relatively unchanged. Mutual elongation of the two adjoining walls of B and C would obviously have involved progressive disappearance, buckling, or a moving upward of the wall of C opposite the progressing tip

of B. There was no evidence of such changes in the wall of C. Rather it must be assumed that the walls of B and C behaved independently of each other, extension being made to one but not to the other. The particular example illustrated relates to growth at the tip of an expanding fusiform initial, but the same principles apply to elongation in the region of overlap between two sister initials.

The other alternative to the old theory of "sliding" growth is that of "intrusive" growth proposed by Sinnott and Bloch (18). They suggested that changes in intercellular relationships involving new cell contacts could come about by sharply localized growth, as for instance at the tips of elongating cells, without slip between the remaining portions of the adjoining walls. While anatomists generally believe growth of elongating cells to be apical, some are uncertain that absolutely no slip accompanies the growth of the intruding tips. For instance, in his report on the development of sclereids Foster (8) has expressed doubt that extension was restricted to the "very tips". As Eames and MacDaniels (7) have stated, distinction depends to some extent on the definition of the "tip" of the elongating cell. With regard to the elongation of fusiform initials in *Chamaecyparis* the authors consider the evidence points to terminal growth, but they are unable to define the precise portion of the tip involved in elongation.

Although their significance is not presently clear, some features of the structure of the tips of tracheids are worth noting. In most rows of tracheids, as viewed in radial section, the ends of successive cells varied in length, shape, and orientation, the differences in length probably being due partly to varying elongation during differentiation (Fig. 11). The pits on the radial faces of these tracheid endings fluctuated in number and arrangement. In number they ranged from few (Fig. 10) to numerous (Fig. 11), though often they were slightly less frequent when the progressive elongation through successive cells was greatest. The arrangement varied, but was usually less regular than on the median portions of the tracheids where an approximate radial alignment was generally apparent. The portion of the tracheid ends with more or less irregular pits ranged from 150μ in cases of moderate elongation to greater extent where elongation was more rapid. Crassulae were often faint or not apparent toward the tip. Probably the increased irregularity in pit arrangement toward the tips of the tracheids was due in part to elongation of the initiating cambial cells and in part to growth during differentiation. The departure from regular radial alignment might be interpreted as indicating that elongation was not confined to the extreme tips of the cells, but present knowledge of the structure of cambial cell walls and the early ontogeny of pit formation do not allow definite conclusions in that regard.

Discussion

In their discussion on growth adjustments, Sinnott and Bloch (18) referred to the problem of the relationship between individual units and the development of the whole cell complex. With regard to fusiform initials in

Chamaecyparis, these exhibited considerable individualism within the limitations imposed upon their behavior by reason of their common association in the cambium. For instance, when one cambial cell perchance elongated only very slowly, a neighbor extended rapidly and by repeated anticlinal divisions gave rise to a fast-growing family of daughter initials. Some initials were reduced in size and disappeared from the cambium, or were segmented into ray initials, when others nearby elongated extensively. Such differences in behavior were noted even among recently formed sister initials. Often the fusiform initials behaved as if competing with one another for more room in the limited space available, some being ever ready to enlarge and to squeeze out a "weaker" neighbor. As a consequence of this competitive activity intercellular adjustments in the cambial zone were more extensive than has been commonly recognized. Instead of the gradual and orderly adjustments assumed by proponents of the "symplastic" theory, sudden changes occurred in the cellular pattern with ensuing rapid alteration in size of individual cells.

Growth of the fusiform initials appeared to be intrusive in the sense that the elongating tips thrust between neighboring initials above and below and forced them apart. The rapidity and amount of elongation seemed to be determined by the interplay between inherent forces within each cell, these varying from one cell to another, and the limitations imposed by the surrounding contacts. For instance, elongating tips were sometimes stalled at obstructing rays, or on the other hand, rapid elongation occurred in association with loss of an adjoining fusiform initial. While growth seemed to be apical, actual extent of the "tip" involved in elongation was not determined. No evidence was found to support the theory of simultaneous elongation over considerable lengths of adjoining walls by "symplastic" growth.

Observations on cambial cell walls reported in the literature have indicated some elaboration of structure with differentiation into primary pit-fields in which plasmodesms are often aggregated (Kerr and Bailey (9)). The implication of such specialization, according to advocates of the "symplastic" theory (Meeuse (11)) is that site of pit development in derived cells is fixed by structure of the cambial cell wall, and that growth adjustments involving slip or intrusion are unlikely because of resulting rupture of plasmodesms and displacement of pit-fields. An incapacity on the part of the intruding tips to form new pit-fields matching those in the stationary walls on either side or to develop plasmodesms is assumed. The evidence indicates that such assumptions are unjustified. It is significant that pits are nearly always more numerous toward the tips of tracheids, in the region of most extensive adjustments, than along the median portions of the cells where longitudinal growth

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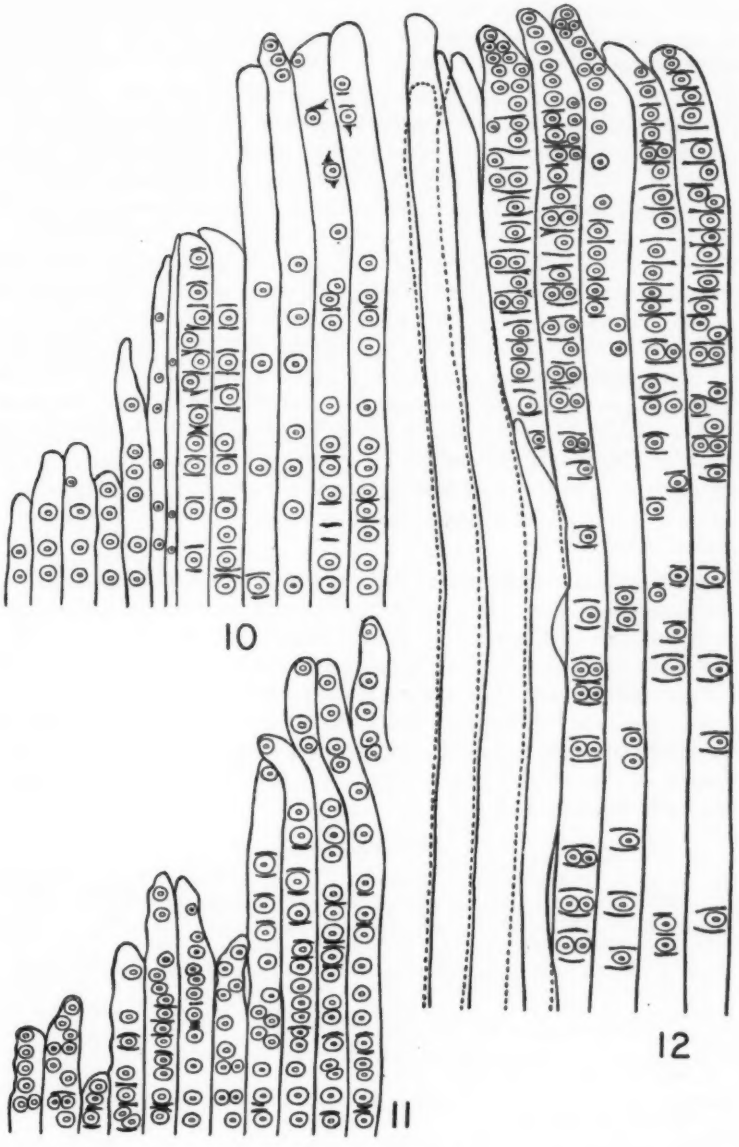
FIGS. 10 AND 11. *Chamaecyparis nootkatensis, old stem. Drawings from radial sections showing number and arrangement of pits at the tips of tracheids derived from lengthening fusiform initials.* FIG. 12. *C. thyoides, old stem. Drawing from radial section showing frequency and arrangement of pits on the newly convergent walls of tracheids which have come together after cessation of the intervening row.*



FIGS. 10 TO 12.

is not occurring. Despite the terminal elongation of fusiform initials and the continued extension of the derived tracheids, the tips of the latter usually have numerous pits. Moreover, the pits coincide with those on the adjoining walls to form pit-pairs.

It is worthy of note that the cessation of an intervening file of cells does not result in pitless walls between the converging tracheids. The frequency of pit formation in the new contact area was studied in some detail with the following results. In some cases the first one or two tracheids arising from the converging fusiform initials, after loss of the intervening cambial cell, had fewer pits along their central portions, the usual number of pits in this area not being attained until the fourth or fifth cell after contact. In other cases there was no obvious impairment of pit formation at any point along the walls (Fig. 12). As shown in this figure a row of tracheids came to an end and the radial walls of the converging files of tracheids developed pits in the usual distribution and frequency immediately after establishment of the new contact. In general, it may be stated that loss of an intervening row of cells sometimes resulted in temporary reduction of pits along the middle portions of the convergent walls, whereas in other instances no change was apparent. Toward the ends, on the other hand, pits were always produced in considerable numbers. In tangential sections these pits were seen to match those on the adjoining walls.

In many cases it is possible that the contacts after intercellular adjustment are between "new" and "old" walls, simplifying the development of pit-pairs. This is probable at the extreme tips of cells. It is doubtful, however, if all the contacts which come into being as a consequence of the addition and loss of cambial cells, and of the growth of the initials and their derived cells are between "new" and "old" walls. It is difficult to avoid the conclusion that the walls of cambial cells are highly adaptive, and that the precise site of pit formation in the derived cells is determined to a large extent by contacts during early maturation.

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HETEROTROPHIC NUTRITION OF SEED PLANTS WITH PARTICULAR REFERENCE TO *LEMNA MINOR* L.[1]

By Paul R. Gorham[2]

Abstract

Lemna minor L. was grown aseptically in darkness for 189 days in a modified Hoagland's solution, containing 1% sucrose, acid-hydrolyzed casein 800 mgm., and yeast extract 40 mgm. per liter as essential ingredients. Etiolated fronds, with root primordia that failed to elongate, were produced at about one-sixth of the rate in light. *Lemna* could not utilize nitrate, ammonium, or urea as heterotrophic nitrogen sources, nor were supplements of acetate, pyruvate, malate, fumarate, succinate, or citrate beneficial in the presence of sucrose. No single amino acid, of 23 tested in a medium containing sucrose plus yeast extract, was as effective as casein hydrolyzate. At suitable concentration, DL-phenylalanine, β-alanine, DL-isoleucine, DL-methionine, and DL-aminobutyric acid were utilized for growth without harmful effects, but other amino acids were either injurious or without effect. Frond development was more normal at low nitrate concentration, but potassium concentration had to be proportionately reduced to avoid inhibition. Growth was not limited by the concentration of other major elements, minor elements, or the degree of aeration. Sucrose feeding and increased photosynthesis had additive rather than competitive effects upon accumulation of growth factors in plants transferred from light to darkness. *Spirodela oligorrhiza* Kurtz. utilized nitrate heterotrophically to a limited extent. Albino maize was grown to the stage of ear formation by feeding sucrose to the leaves and nitrate to the roots. Growth was not promoted, however, by corn seed diffusate, coconut milk, or ammonium plus casein hydrolyzate supplied to the leaves. Albino barley fed sucrose through the leaves did not grow beyond the seedling stage. The heterotrophic requirements reported for various plants, plant organs, and tissues are compared, and the existence of two mechanisms of nitrate assimilation, one of which requires light, is discussed.

Introduction

Most plants may be classified, with respect to nutrition, as autotrophs or heterotrophs. The former utilize light and grow without dependence on an external supply of organic substances. The latter are unable to utilize light, but derive energy from suitable organic sources of carbon. They frequently require an organic source of nitrogen as well. By feeding suitable compounds it has been found possible to grow a number of autotrophs in darkness as heterotrophs. Likewise, many albino or colorless forms have been cultured successfully.

Probably most, if not all, plants, plant organs, and tissues are capable of heterotrophic growth when fed the compounds that are normally supplied by photosynthetic and allied processes. To find out something about the nature and function of these compounds has been the purpose of most such nutritional investigations. As early as 1887, Klebs (40) reported the culture of the alga

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Zygnema in darkness for six months on a medium containing glucose, and noted, moreover, that it remained green. Since then, many algae and flagellate protozoa have been cultured heterotrophically, some producing chlorophyll in darkness (20, 23, 42, 59), others not. Heterotrophic culture of the mosses, ferns, and seed plants has been investigated less extensively since they grow slowly – often in a manner which makes the supplying of necessary organic substances difficult. Much work has been done, however, on the nutritional requirements of isolated embryos, organs, and tissues of the higher plants grown in darkness as well as in light.

Early attempts to culture seed plants heterotrophically by germinating seeds of albino maize (41) or albino *Artemisia* (63) on sterile nutrient agar containing sucrose were only partially successful. A few leaves were produced, but the plants failed to mature. By feeding sucrose through the cut leaf tips instead of the roots, Spoehr (74) succeeded in growing albino maize to maturity in diffuse light, using a nutrient solution containing nitrate, which was preferable to ammonium as the source of nitrogen. Both albino and green maize plants grown in the dark exhibited elongation and other symptoms typical of etiolation. The low growth rate of the albinos could not be accelerated by additions of various vitamins, adenine, or indolylacetic acid to the sucrose solution.

In a previous report (27), it was shown that the aquatic duckweed, *Spirodela oligorrhiza*,* could utilize nitrate plus sucrose for limited growth when transferred to darkness. In experiments that lasted from 14 to 21 days, the number of etiolated fronds produced was increased when asparagine was added to the medium, but this seemed to depend on whether the plants used for inoculum had been grown with sucrose in the light. Cystine plus methionine and aspartic but not glutamic acid also increased the rate of frond multiplication. Other amino acids and various vitamins had no effect and ammonium sulphate was inhibitory.

A new investigation was undertaken using the related species, *Lemna minor* L., to determine in greater detail which nitrogen sources are utilized in darkness, with the purpose of devising a medium that would support growth indefinitely, and perhaps of identifying or overcoming metabolic factors that restrain growth and induce other changes. The nutrient requirements for growth of albino maize to a mature stage were also investigated.

Materials

Experimental Plants

The duckweed used for these experiments was *Lemna minor* L., chosen in preference to *Spirodela* spp. because developing fronds show less overlapping and counting is thereby facilitated. *Lemna* plants were obtained from a

* *The plant was called Spirodela polyrrhiza (L.) Schleid, but subsequent taxonomic investigations indicate that the species employed was probably S. oligorrhiza Kurtz.*

local pond and a clone was started from a single frond freed of contaminating microorganisms by ethanol – mercuric chloride treatment (27).

A comparison culture of *Spirodela oligorrhiza* was obtained through the courtesy of Dr. Yvette H. Edmondson, Biological Laboratories, Harvard University. Seeds of a strain of maize segregating albinos as a simple recessive were obtained through the courtesy of Dr. Rosalind Morris, Department of Plant Breeding, Cornell University. Seeds of striped barley from lines yielding a high percentage of albinos (4) were kindly furnished by Dr. T. J. Arnason, Department of Biology, University of Saskatchewan. Sufficient seeds of maize and barley for the present experiments were produced by careful propagation in the field.

Chemicals and Test Substances

All inorganic chemicals were of standard reagent grade. Vitamins, amino acids, amides, purines, and pyrimidines were obtained from General Biochemicals Inc., Eastman Kodak Co., Merck and Co., Inc., or Schwarz Laboratories, Inc. Acid-hydrolyzed casein, vitamin-free, was obtained from General Biochemicals, and yeast extract, peptone, and tryptone from Difco Laboratories, Inc. The liver extract was Wilson Laboratories' L-20.

Corn seed diffusate was prepared by soaking 2 lb. of Golden Bantam seed in distilled water in a cheesecloth bag for four days at 21° C. The seeds were dipped and drained repeatedly. Each day the water was renewed, the diffusate stored at 0° C., and finally pooled, adjusted to pH 4.8, and sterilized by Seitz filtration in lots of 200 ml.

The milk of six coconuts (500 ml.) was filtered through Whatman No. 12 paper, adjusted to pH 4.8, and aliquots of 50 ml., sterilized by Seitz filtration, added to 150-ml. lots of sterile, 13.3% sucrose solution. Dilute coconut milk was used for albino maize. The milk of two coconuts, similarly treated but undiluted, was used for experiments with *Lemna*.

Two growth factor supplements, referred to as X and Y, used in the albino maize experiments, contained the following ingredients, adjusted to pH 4.8 and autoclaved at 15 lb. for 20 min.:

	X	Y
Sucrose	100.0 gm.	100.0 gm.
Casein hydrolyzate	1.0 gm.	0.8 gm.
Adenine sulphate	0.1 mgm.	0.1 mgm.
Guanine hydrochloride	0.1 mgm.	0.1 mgm.
Xanthine	0.1 mgm.	0.1 mgm.
Uracil	0.1 mgm.	0.1 mgm.
Yeast extract	40.0 mgm.	40.0 mgm.
Asparagine	100.0 mgm.	100.0 mgm.
L-tryptophan	—	40.0 mgm.
Indolylacetic acid	1.0 mgm.	—
M ammonium sulphate	1.0 ml.	—
1/5 Hoagland's solution	1.0 liter	—
Distilled water	—	1.0 liter

Methods

Light Culture of Lemna

The culture conditions used previously (27) were modified as follows: 1. The manganese concentration of Hoagland's nutrient was doubled.* 2. Larger test tubes (29 × 180 mm.) were employed, supported in cadmium-plated racks that held them erect for inoculation and inclined 80 degrees for culture. 3. Light was furnished by three 40-w. "White" fluorescent lamps, with reflector, adjusted to produce an intensity of 250 ft-c. 4. The temperature was 21 ± 0.3° C.

Two lines of stock cultures were maintained by weekly transfers of 20 or 25 fronds to fresh, sterile media with or without 2.5% sucrose, respectively. The remaining plants after subculture, numbering some 175 fronds per tube, were used for experiments within the next three days – well before overcrowding caused signs of yellowing. In three experiments, the cultures were aerated or treated with tank mixtures of either 5% carbon dioxide in air or 5% carbon dioxide in nitrogen by means of capillary aerators connected in series as previously described (27).

Dark Culture of Lemna

Sterile solutions were prepared in large test tubes just as for light culture. The racks containing the tubes were kept in total darkness at 25 ± 0.2° C. Green safelights (27) were used, for as short a time as possible, in examining or transferring the cultures. When not being handled, cultures were kept several feet from these safelights.

All treatments were in triplicate. Each tube of medium was inoculated with 50 fronds from the light-grown cultures. The average number of fronds per tube was determined at intervals from counts made over a period of two to three weeks. To obtain a numerical expression of the rate of frond multiplication for a particular interval, the slope of the line K (19), given by the logarithm of frond number plotted against time in days, was determined. For convenience, this index was multiplied by 1000, and called the "multiplication rate" or "MR" for a stated interval.

At the conclusion of an experiment, the plants were collected on a wire screen, washed well with distilled water, and dried in aluminum weighing cans with a current of air under a battery of infrared lamps for four hours at 40° C. After 16 to 24 hr. in a desiccator, the cans were weighed and the results expressed as dry weight per 100 fronds.

Analyses for sugars and starch were carried out on about 100 mgm. of dried tissue, ground to 40 mesh in a micro-Wiley mill. Reducing and non-reducing sugars were extracted with 80% ethanol, and the residue was hydrolyzed with takadiastase. Sucrose was hydrolyzed with invertase. Hassid's

** Hoagland's nutrient as used contained: $Ca(NO_3)_2.4H_2O$ 5, $MgSO_4.7H_2O$ 2, KNO_3 5, KH_2PO_4 1 mM. per liter; and $Fe_2(C_4H_4O_6)$ 5, H_3BO_3 2.86, $MnCl_2 . 4H_2O$ 3.62, $ZnSO_4 . 7H_2O$ 0.22, MoO_3 0.07, $CuSO_4 . 5H_2O$ 0.08 mgm. per liter; pH 4.8.*

ferricyanide procedure (33) was used for the determination of reducing sugars. Conversion factors of 0.95 and 0.90 were used for sucrose and starch, respectively.

Culture of Albinos

Maize seeds were sprouted in soil. After two weeks, albinos having two leaves of good size were transplanted to 4-in. pots of soil and feeding of the lower leaf with 10% sucrose was begun, following Spoehr's procedure (74). The feeding leaves were clipped and the vials of solution changed at least three times weekly. New leaves were fed when they had elongated sufficiently. Old leaves were no longer fed when they became too short for immersion. After about two more weeks, when the death of the unfed check plants signalled the exhaustion of seed reserves, the youngest feeding leaves were treated with test solutions while the older leaves continued to receive sucrose. For growth in nutrient solution instead of soil, the seedlings were germinated in vermiculite, transplanted to quartz sand, and subirrigated four times daily. Albino barley seedlings, germinated in soil, were transplanted to 2-in. pots and fed through the leaves. The maize and barley plants were grown in a greenhouse section screened to keep out direct sunlight. The temperature was 20 ± 1° C. and the relative humidity 40 ± 10%.

Results

Sustained Growth of Lemna in Darkness

The first experiments were designed to find out whether *Lemna* would grow on a nitrate medium to which various sugars had been added. Sucrose and dextrose were compared over a range of concentrations from 0 to 2.5%. A small increase in frond number was observed but this was not dependent on the addition of either sugar to the medium (Table I). Both sugars caused a pronounced increase in frond dry weight that varied with the concentration. By testing with iodine, the treated fronds were found to be gorged with starch. Fructose, corn syrup, honey, and cane molasses were no more satisfactory as carbohydrate sources than dextrose or sucrose, and the magnesium salt of fructose diphosphate at a concentration of 0.5% was toxic.

Purines and pyrimidines, yeast extract, and coconut milk have been shown to promote the growth of leaves, stems, or excised embryos (9, 10, 52, 84). A medium was prepared containing 2.5% sucrose and adenine sulphate, guanine hydrochloride, xanthine, uracil, yeast extract, casein hydrolyzate, and asparagine in what were thought to be nontoxic concentrations. The effect of a Seitz-filtered preparation of fresh coconut milk was also tested. The dried samples were analyzed for sugars and starch at the conclusion of the experiment (Table II). Casein hydrolyzate produced a small but definite increase in frond multiplication rate which was greater than that produced by coconut milk. A few fronds were produced that were entirely etiolated. Analysis confirmed the magnitude of starch accumulation, and showed that reducing sugars and disaccharides were present in the same quantities as in

TABLE I

EFFECT OF VARIOUS SUGAR CONCENTRATIONS UPON THE RATE OF FROND MULTIPLICATION AND FROND DRY WEIGHT OF *Lemna* GROWN IN DARKNESS

Concentration, %	Sucrose		Dextrose	
	MR (0-14 days)	Dry wt. per 100 fr., mgm.	MR (0-14 days)	Dry wt. per 100 fr., mgm.
0	4	16	6	16
0.5	7	27	6	27
1.0	6	31	6	31
1.5	7	30	4	34
2.0	7	34	7	35
2.5	5	35	6	37

TABLE II

GROWTH RATE OF *Lemna* IN DARKNESS IN MIXTURES OF GROWTH PROMOTING SUBSTANCES

Medium	MR (0-14 days)	Dry wt. per 100 fr., mgm.	Glucose, %	Sucrose, %	Starch, %
Coconut milk*	9	49	2.8	9.5	45.3
Growth factors**	13	39	1.9	6.5	47.1
Control	5	31	2.4	5.1	47.3

** Contained 2.5% sucrose plus coconut milk 2.5 ml. per 25 ml. of Hoagland's nutrient.*

*** Contained 2.5% sucrose plus casein hydrolyzate 400 mgm., adenine sulphate 0.1, guanine hydrochloride 0.1, xanthine 0.1, uracil 0.1, yeast extract 40, and asparagine 100 mgm. per liter of Hoagland's nutrient.*

light-grown plants. The amounts of sugar and starch did not appear to be appreciably affected by the rate of frond multiplication. Increasing the concentration of casein hydrolyzate or asparagine five times caused growth inhibition accompanied by a general browning and a marginal necrosis of the fronds.

The importance of frond dry weight as a criterion of growth diminished with the observation that a large part of the change was caused by starch synthesis in response to carbohydrate feeding. Accordingly, more attention was paid to the rate of frond multiplication.

The possibility that yeast extract might not be supplying a sufficient amount of some particular growth factor was investigated. A supplement containing 13 vitamins or other growth substances was added to a medium containing 2.5% sucrose plus casein hydrolyzate, adenine, guanine, xanthine, uracil, and calcium carbonate. The pH was adjusted to 5.3 in order to leave some carbonate undissolved. Ammonium sulphate, 1 gm. per liter, and indolylacetic acid, 2 mgm. per liter were tested at the same time. The vitamin supplement was slightly inhibitory (Table III). Ammonium sulphate

TABLE III

GROWTH RATE OF *Lemna* IN DARKNESS IN THE PRESENCE OF SPECIFIC GROWTH PROMOTING SUBSTANCES

Treatment	MR (0-14 days)
Vitamin supplement, ammonium sulphate, and indolylacetic acid*	10
Vitamin supplement	10
Ammonium sulphate	13
Indolylacetic acid	14
Basal medium**	13

** Final concentration: ascorbic acid 2, thiamine 2, riboflavin 2, pyridoxine 2, p-aminobenzoic acid 1, calcium pantothenate 2, niacin 1, inositol 10, choline 4, yeast nucleic acid 4, and asparagine 100 mgm. per liter, plus biotin 0.4 and pteroylglutamic acid as "Folvite" 0.4 µgm. per liter. Ammonium sulphate 1 gm. per liter, indolylacetic acid 2 mgm. per liter.*

*** Contained 2.5% sucrose, casein hydrolyzate 400, adenine sulphate 0.1, guanine hydrochloride 0.1, xanthine 0.1, uracil 0.1, and calcium carbonate 400 mgm. per liter of Hoagland's nutrient.*

caused the inoculum fronds to become slightly brown, but did not affect growth appreciably. Indolylacetic acid caused epinasty and clumping of the fronds but did not increase the rate of multiplication.

The effect of substituting liver extract, peptone, or tryptone for yeast extract (40 mgm. per liter) in a medium containing casein hydrolyzate 400 mgm., adenine sulphate 0.1, guanine hydrochloride 0.1, xanthine 0.1, uracil 0.1, and asparagine 100 mgm. per liter was tested with a sucrose concentration of 2.0%. Over a test period of 20 days, yeast extract gave the highest rate of frond multiplication. The addition of L-tryptophan (10 mgm. per liter) to the medium with yeast extract, to replace losses caused by acid hydrolysis of casein, produced slightly larger fronds but did not affect multiplication rate.

The optimal sucrose concentration was determined with the yeast extract and tryptophan medium of the preceding experiment. Subcultures were made at the end of 21 days and the experiment concluded at the end of a growth period of 43 days. In Fig. 1, the increase in frond number with time is plotted for the cultures with 1% sucrose. During the first two days, the initially high rate of frond multiplication declined, and was followed by a period of several days in which little or no growth occurred. Also, during this time, the roots of the inoculum fronds died. Gradually, a low but steady rate of frond multiplication was established with the new fronds lacking chlorophyll and bearing root primordia that failed to elongate.

A similar sequence of events occurred at other sucrose concentrations. The effects on the rate of frond multiplication and on frond dry weight are summarized in Tables IV and V. There was little or no growth in the absence

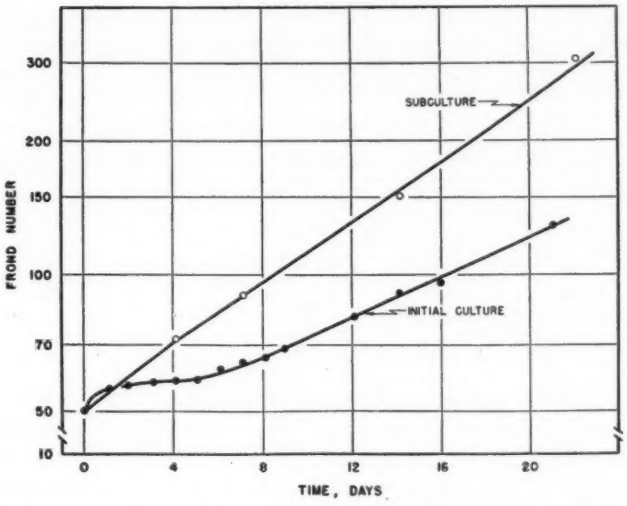

FIG. 1. *Average increase in frond number with time of Lemna grown in darkness in a medium containing 1% sucrose plus casein hydrolyzate 400, adenine sulphate 0.1, guanine hydrochloride 0.1, xanthine 0.1, uracil 0.1, yeast extract 40, asparagine 100, and L-tryptophan 10 mgm. per liter.*

TABLE IV

THE EFFECT OF INCREASING SUCROSE CONCENTRATION UPON GROWTH OF *Lemna* IN DARKNESS

Sucrose,* %	MR							Dry wt. per 100 fr., mgm.	
	Culture				Subculture			Culture, 0-21 days	Subculture, 21-43 days
	Interval, days								
	0-2	2-7	7-14	14-21	21-25	25-28	28-43		
0	40	0	2	0	3	0	2	17	19
0.5	25	6	19	20	30	28	33	35	31
1.0	29	13	19	22	40	32	36	44	33
1.5	47	5	19	20	50	38	36	47	38
2.0	29	9	18	24	65	31	37	51	36
2.5	25	4	16	21	65	33	37	56	36

* *In the same basal medium as given with Fig. 1.*

of sucrose (Table IV), showing that casein hydrolyzate and the other nitrogen-containing compounds are unsuitable as carbon sources. Subculture temporarily stimulated multiplication rate. Fronds were more normal in size and appearance with 0.5 to 1.0% sucrose than with higher concentrations and, except for the subculture effect, they multiplied at about the same rate.

TABLE V

THE EFFECT OF DECREASING SUCROSE CONCENTRATION UPON GROWTH OF *Lemna* IN DARKNESS

Sucrose,* %	MR		Dry wt. per 100 fr., mgm.
	Interval, days		
	7-14	14-21	
0.50	18	22	35.4
0.25	14	20	33.4
0.10	16	10	29.1

* *In the same basal medium as given with Fig. 1.*

Starch accumulation was so great at high sucrose concentrations that many plants sank to the bottom of the tubes. The size of new fronds tended to decrease as the experiment progressed. The effect of reducing the sucrose concentration to 0.1% was to diminish both multiplication rate and dry weight (Table V), so 1.0% was considered optimal.

Increasing the concentration of L-tryptophan as much as fivefold or the concentration of asparagine twofold had no effect. Doubling the concentration of casein hydrolyzate increased the rate of frond multiplication and reduced frond dry weight slightly. The following formula, which produced a slow growth in darkness, was adopted as a standard or reference supplement for further experiments:

Substance	Code	Mgm. per liter
Casein hydrolyzate	C	800
Adenine sulphate, guanine hydrochloride, xanthine, uracil	P	0.1 each
Yeast extract	Y	40
Asparagine	A	100
L-tryptophan	T	10

The code letters, singly or combined, will be used hereafter to designate supplements of these ingredients in the stated amounts. With abbreviations for Hoagland's nutrient (H), or a modification (L) (potassium and nitrate both at 1×10^{-3} *M* instead of at 6 and 15×10^{-3} *M*, respectively) and by specifying the amount of sucrose (S), the composition of different media will be expressed by formulae such as 1%SH, CPYAT; 1%SL,CY; etc.

The ability of 1%SL,CPYAT medium to sustain growth over a long period, through several subcultures, was tested. The essentiality of PAT and Y was also determined. Starting at about the 32nd day, the cultures with only

casein hydrolyzate declined in vigor and by the 90th day were dead (Table VI). Except for the tendency to increase following subculture, the multiplication rate was reasonably steady and similar with CY for a period of 109

TABLE VI

EFFECTS OF THREE TYPES OF MEDIA ON THE PROLONGED DARK CULTURE OF *Lemna*

Sub-culture No.	Interval, days	Media					
		1%SL,CPYAT		1%SL,CY		1%SL,C	
		MR	Dry wt. per 100 fr., mgm.	MR	Dry wt. per 100 fr., mgm.	MR	Dry wt. per 100 fr., mgm.
	0-3	57	—	61	—	66	—
	3-11	12	—	17	—	16	—
0	11-14	16	—	17	—	21	—
	14-21	18	—	25	—	24	—
	21-24	21	49.4	21	40.4	16	39.6
	24-27	53	—	40	—	31	—
1	27-32	23	—	28	—	22	—
	32-44	7	—	17	—	14	—
	44-50	17	41.2	17	44.2	7	40.2
	50-53	45	—	22	—	6	—
2	53-62	23	—	20	—	4	—
	62-90	—	—	—	—	Dead	—
3	90-109	26	39.1	27	42.0	—	—
4	109-189	31	—	—	—	—	—

days, and with CPYAT for 189 days. Frond size did not alter but no roots developed. The frond dry weight with CPYAT decreased somewhat. Casein hydrolyzate and yeast extract were essential for sustained growth but adenine, guanine, xanthine, uracil, and L-tryptophan were not.

Other Carbon and Nitrogen Sources

A number of other compounds were tested for their ability to increase the sustained growth rate obtainable with 1%SH,CPYAT. Sodium acetate at a concentration of 1% proved toxic, while 0.1% D-ribose or 100 mgm. per liter of inositol had no effect either upon frond multiplication rate or dry weight.

Although respiratory intermediates are utilized by some facultative heterotrophs, pyruvate, malate, fumarate, succinate, and citrate, tested in 1%SL,CPYAT at a concentration of 0.5×10^{-3} *M*, had no growth promoting effects. Concentrations 5 to 10 times greater were inhibitory or toxic.

Doubling the concentration of yeast extract or adding 40 to 160 mgm. per liter yeast nucleic acid to a 1%SL,CY medium had no effect upon growth.

Urea, at concentrations of 10^{-2} and 2×10^{-3} *M* proved toxic both in the presence and absence of CPYAT. When ammonium was substituted for nitrate in 1%SH,CPYAT, as little as 1.5×10^{-3} *M* was toxic.

Nineteen naturally occurring amino acids and four synthetic amino acids were tested in a medium containing sucrose and yeast extract (2.5%SH,Y) using 2.5%SH,CPYAT as a reference treatment to relate individual experiments. Each of the naturally occurring acids was tested at a concentration approaching that provided by 800 mgm. per liter of casein hydrolyzate, based on estimates derived from published analyses (28; 78; 79; 92, p. 81). Nine amino acids of particular interest were tested at higher or lower concentrations. The plants grew very slowly in the basal medium as compared with the medium containing CPYAT (Table VII). No single amino acid was as

TABLE VII

EFFECT OF INDIVIDUAL AMINO ACIDS IN 2.5%SH,Y MEDIUM ON GROWTH OF *Lemna* IN DARKNESS

(Average MR (14-21 days) for basal medium 4, for CPYAT 28)

Amino acid	Conc., mgm./liter	MR (14-21 days)	Symptoms**	Amino acid	Conc., mgm./liter	MR (14-21 days)	Symptoms**
DL-Phenylalanine	100	15		DL-Methionine	20*	14	
	250	14			40	17	
	500	9					
	1000	4		DL-Threonine	25	11	N
					50*	18	
β-Alanine	20*	16					
	250	0	D	L-Proline	25	14	N
					50*	9	
DL-Alanine	20*	6					
	250	3	D	DL-Aminobutyric	100	12	
				L-Cystine	5	9	
DL-isoleucine	100*	11		L-Tryptophan	20	9	N
	250	12		L-Lysine	50	8	N
	1000	17		L-Tyrosine	50	7	D
				L-Glycine	5	7	
L-Leucine	50*	12	N	L-Histidine	20	6	
	125	2	N	L-Hydroxyproline	5	5	
	500	0	N	DL-Aspartic	100	5	
				DL-Serine	100	4	D
DL-Valine	100*	7		DL-Glutamic	100	2	N
	250	0	N	L-Arginine	20	2	
				DL-Aminocaproic	100	0	D
				DL-Aminovaleric	100	0	D

* *Concentrations tested initially.*
** *N = necrotic spots. D = dead.*

effective as CPYAT, although in suitable concentration, DL-phenylalanine, and β-alanine but not DL-alanine, DL-isoleucine, DL-methionine, and DL-aminobutyric acid were utilized for increased growth without harmful symptoms. Over the range of concentrations tested, L-leucine, DL-threonine, and L-proline stimulated growth but produced symptoms of necrosis, particularly in the fronds and roots of the original inoculum. Of the remaining acids, L-tyrosine, DL-serine, DL-aminocaproic, and DL-aminovaleric were toxic at rather low concentrations; L-tryptophan, L-lysine, and DL-glutamic acid produced symptoms of necrosis, while the others produced no effects.

Mineral Nutrition and pH

Some experiments were carried out to ascertain what effects, if any, changes in concentration of the major and minor elements would have on growth, using 1%SH,CPYAT as the reference medium. The interaction of potassium at 0, 6, and 12×10^{-3} *M* and of nitrate at 0, 1.5, and 15×10^{-3} *M* was studied, using potassium chloride, sodium nitrate, and disodium phosphate in place of the usual potassium nitrate and potassium phosphate. The rate of frond multiplication was but little affected until the third week. In that period, however, the rate declined in the cultures containing potassium that were low in nitrate, but remained unchanged in the cultures with 15×10^{-3} *M* nitrate regardless of the potassium concentration (Table VIII). The plants

TABLE VIII

EFFECT OF POTASSIUM AND NITRATE CONCENTRATION ON THE GROWTH OF *Lemna* IN DARKNESS ON 1%SH,CPYAT MEDIUM

(Figures in parentheses for Hoagland's medium having no sodium)

Growth criterion	Nitrate, 10^{-3} *M* per liter	Potassium, 10^{-3} *M* per liter		
		0	6	12
MR (14-21 days)	0	24	13	11
	1.5	26	14	10
	15	23	23 (23)	21
Dry wt. per 100 fr., mgm.	0	36	42	42
	1.5	36	43	45
	15	42	43 (42)	45

grown without potassium and with low nitrate showed no decline in multiplication rate, and had larger fronds that closely resembled those grown in light except, of course, for the lack of green pigments and roots. The dry weight of these fronds was slightly lower, but the sugar and starch composition was found to differ very little from that of the controls.

A study was made of the effects of varying the initial hydrogen ion concentration of the medium from pH 4.8 to 7.0 and of varying the phosphate concentration from 1 to 5×10^{-3} *M* in the hope of being able to regulate the

amount of starch produced (32). Growth was similar in all cultures and all had a final pH of 5.6. An initially high pH and high phosphate concentration externally had no effect upon starch deposition internally.

Interaction between magnesium, calcium, and sulphate was investigated by means of the triangle method. The concentrations used were: magnesium 0, 2, 4, and 6; sulphate 0, 2, 4, and 6; and calcium 0, 1, 3, 5, and 8×10^{-3} *M*. A necrosis of the fronds appeared in the cultures lacking calcium. Growth of the remaining cultures was similar to that of the controls.

Omission of both ferric tartrate and the supplement of minor elements, doubling the concentration of these substances, or increasing the manganous ion concentration up to 15 times had no effects upon growth in 1%SL,CPYAT medium.

The principal difference between the mineral nutrition of *Lemna* in heterotrophic as compared with autotrophic culture was that nitrate, which no longer served as a suitable nitrogen source, had a slight inhibitory effect upon development and growth. When the nitrate concentration was reduced, the potassium concentration also had to be reduced to avoid growth inhibition. Calcium deficiency was readily expressed by symptoms of necrosis. Varying the concentration of the other elements within reasonable limits made very little difference as far as growth with 1%SH,CPYAT was concerned. A modification of Hoagland's nutrient, called *Lemna* medium (L), was therefore adopted, containing potassium and nitrate both at 1×10^{-3} *M* instead of 6 and 15×10^{-3} *M*, respectively.

Aeration

The possible effects of aeration or oxygenation on the rate of growth were investigated by culturing in gas-washing bottles each containing 100 ml. of 1%SH,CPYAT. A slow stream of sterile, washed air was passed through the sintered glass aerators of three bottles connected in series. The control cultures were grown in 100 ml. of medium in 200-ml. Erlenmeyer flasks. Better aeration by frequent transfer to fresh medium under the usual culture conditions in test tubes was investigated at the same time. One series was transferred to fresh medium twice and another series five times during the growth period of 21 days. The results of these tests (Table IX) showed that

TABLE IX

GROWTH OF *Lemna* IN DARKNESS AS AFFECTED BY AERATION AND FREQUENT TRANSFER

Treatment	MR (14-21 days)	Dry wt. per 100 fr., mgm.
Aerated	23	31.2
Unaerated	22	36.9
Transferred five times	28	38.2
Transferred twice	25	37.7
Untransferred	22	38.8

multiplication rate was unaffected by aeration, although frond weight was somewhat diminished. Frequent transfer promoted growth slightly, but this response to fresh media, noted also in connection with long-term culture (Table VI), is evidently not related to the degree of aeration. When pure oxygen was bubbled into the culture flasks under sterile conditions, plants on 1%SL,CPYAT grew for only a few days and then died.

Light-pretreatment Effects

It appeared from experiments with *Spirodela* (27) that substances produced by the plants in light, and accumulated to an appreciable degree only when sucrose was supplied, were essential for even limited growth in darkness. An "inoculum effect" when *Lemna* fronds grown on 2.5%SH medium in light were transferred to 1%SH,CPYAT medium in dark has already been noted (Fig. 1). With the object of gaining more information about the inoculum effect, *Lemna* plants were given pretreatments calculated to increase or decrease the supply of these hypothetical substances. The effects were measured by the frond multiplication rate during the first few days in darkness on 1%SH,CPYAT medium.

Cultures inoculated with 15 fronds were grown at 570 ft-c. on the regular 2.5%SH and also on 1%SH,CPYAT media, both with and without aeration. The rate of frond multiplication in light was measured. Subcultures, starting with 50 fronds, were then grown in darkness. *In light*, 1%SH,CPYAT produced fronds that were larger and greener with longer roots than did 2.5%SH; aeration diminished frond size, root length, and dry weight (Table X). *In dark*, all new fronds were of medium size and had similar dry weights

TABLE X

GROWTH OF *Lemna* AND FROND APPEARANCE AFTER PRETREATMENT IN LIGHT FOR FIVE DAYS

Medium	Treatment	Frond size	Frond color	Relative root length	MR (0-6 days)	Dry wt. per 100 fr., mgm.
2.5%SH	Unaerated	Large	Light green	1.0	160	24.0
	Aerated	Medium	Green	0.5	143	10.3
1%SH,CPYAT	Unaerated	Very large	Shiny, deep green	1.3	188	24.3
	Aerated	Large	Dull green	0.3	182	14.6

(40 mgm. per 100 fronds). *In light*, the rate of frond multiplication was greater on 1%SH,CPYAT than on 2.5%SH, but aeration tended to diminish it slightly (Table X and Fig. 2). *In dark*, plants from 2.5%SH produced the greatest inoculum effect (Fig. 2) followed by practically no lag phase. The effect of both aeration and 1%SH,CPYAT pretreatment was a decreased inoculum effect and pronounced lag phase that lasted for six days or longer.

During the final week in darkness, all cultures multiplied at substantially the same low rate – at its best only about one-sixth the rate in light on the same medium.

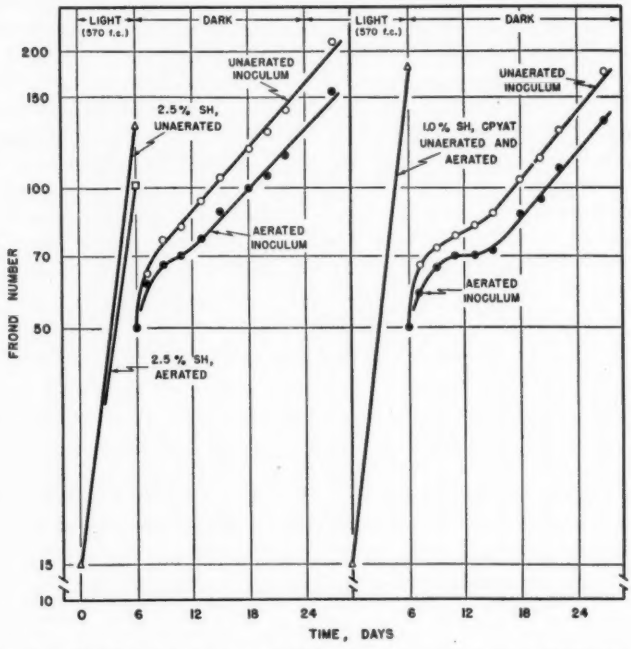

FIG. 2. *The effect of pretreatment in light upon subsequent frond multiplication of Lemna in darkness on 1% SH,CPYAT medium.*

In a second experiment, the inocula were taken from H and 2.5%SH media, aerated and unaerated. The results, summarized in Table XI, showed again that aeration tended to reduce the rate of frond multiplication and frond dry weight in light and to cause a decided decrease in the inoculum effect. The inoculum effect (0 to 1 day) was greatest and the lag phase (1 to 7 days) least apparent with fronds from medium containing sucrose. The inoculum effect was least and the lag phase most apparent with fronds from aerated media without sucrose. As before, growth in darkness after the first seven days was similar in all cultures. Final frond weights were also similar.

To ascertain whether the carbohydrate content of the fronds largely determined the magnitude of the inoculum effect, plants from H medium were transferred to media containing 0, 2.5, 5.0, and 10.0% sucrose and allowed to grow and accumulate sugar for two days at 250 and 500 ft-c., then subcultured and grown in darkness for 22 days. Sucrose accumulation decreased frond multiplication at both light intensities, a concentration of 10% stopping growth altogether (Table XII). The inoculum effect (0 to 1 day) was greater

TABLE XI

GROWTH OF *Lemna* IN LIGHT AND IN DARKNESS AS AFFECTED BY SUCROSE AND AERATION PRETREATMENTS

Light, 530 ft-c.			Dark						
Pretreatment	MR (0-5 days)	Dry wt per 100 fr., mgm.	MR						Dry wt. per 100 fr., mgm.
			Interval, days						
			0-1	1-2	2-4	4-7	7-14	14-21	
2.5%SH	169	22.9	124	28	17	18	19	24	38.8
2.5%SH, aerated	163	13.1	74	24	7	13	21	20	40.5
H	160	15.4	60	39	11	9	22	22	41.4
H, aerated	146	11.0	45	9	7	10	24	21	41.3

TABLE XII

GROWTH OF *Lemna* IN LIGHT AND IN DARKNESS AS AFFECTED BY SUCROSE ACCUMULATION AT TWO LIGHT INTENSITIES

Light			Dark					
Intensity, ft-c.	Sucrose concentration, %	MR (0-2 days)	MR					
			Interval, days					
			0-1	1-2	2-4	4-7	7-14	14-22
250	0	90	83	38	12	10	19	18
	2.5	67	104	54	14	16	20	21
	5.0	36	68	40	26	25	22	26
	10.0	0	44	11	37	45	18	25
500	0	103	111	27	14	16	21	18
	2.5	77	118	60	13	17	20	22
	5.0	36	98	54	27	26	21	25
	10.0	0	52	11	37	31	13	20

with pretreatment at 500 ft-c. than at 250 ft-c. for all sucrose concentrations; the optimal concentration in each case was 2.5%. The inoculum effects were lower with 5% and 10% sucrose than with no sucrose pretreatment. Five percent sucrose pretreatment eliminated the lag phase (1 to 7 days), while 10% caused a brief lag phase followed by a phase in which frond multiplication was exceptionally high with another less pronounced lag from the 7th to the 14th days.

Even when plants were grown at 700 ft-c., pretreatment with 2.5% sucrose increased the inoculum effect (measured this time from 0 to 4 days) and diminished the lag phase as compared to the control without sucrose (Table XIII). Increasing photosynthesis with 5% carbon dioxide in air, in the presence of sucrose, caused an increased inoculum effect without any change

TABLE XIII

GROWTH OF *Lemna* IN LIGHT AND IN DARKNESS AFTER PRETREATMENT IN LIGHT WITH CARBON DIOXIDE IN THE PRESENCE OR ABSENCE OF SUCROSE

Light, 700 ft-c.				Dark			
				MR			
Medium	Treatment	MR (0-7 days)	Dry wt. per 100 fr., mgm.	Interval, days			
				0-4	4-7	7-15	15-21
H	—	164	15.6	24	11	17	33
	5% CO_2 – air	171	24.3	35	19	32	32
	5% CO_2 – N_2	141	43.3	41	16	31	38
2.5%SH	—	177	18.9	45	26	24	28
	5% CO_2 – air	176	26.0	52	23	29	38
	5% CO_2 – N_2	152	31.1	64	19	26	36

in the lag phase. With 5% carbon dioxide in nitrogen, the inoculum effect was further increased but there was a lag phase from the fourth to the seventh days.

Summarizing these results, the inoculum effect was increased by pretreatments in light such as sucrose feeding, increased light intensity, and an enriched supply of carbon dioxide in air or in nitrogen, but was decreased by aeration, or by accelerated growth in response to CPYAT feeding.

Growth of Spirodela in Darkness

A culture of *Spirodela*, having a multiplication rate in light on 2.5%SH of 111 as compared with 132 for *Lemna* under the same conditions, was used to inoculate 0, 0.5, and 2.5%SH media, with and without CPYA. Neither species grew significantly without sucrose or with only CPYA (Table XIV).

TABLE XIV

COMPARATIVE GROWTH IN DARKNESS OF *Lemna* AND *Spirodela*

Medium	*Lemna minor*			*Spirodela oligorrhiza*		
	MR		Dry wt. per 100 fr., mgm.	MR		Dry wt. per 100 fr., mgm.
	Interval, days			Interval, days		
	7-14	14-21		7-14	14-21	
H	0	1	9.7	1	0	15.3
0.5%SH	1	4	35.2	1	4	22.7
2.5%SH	1	2	45.3	2	11	26.7
H,CPYA	0	0	12.1	3	1	15.9
0.5%SH,CPYA	11	23	34.5	12	21	22.1
2.5%SH,CPYA	13	16	68.8	24	30	26.3

PLATE I

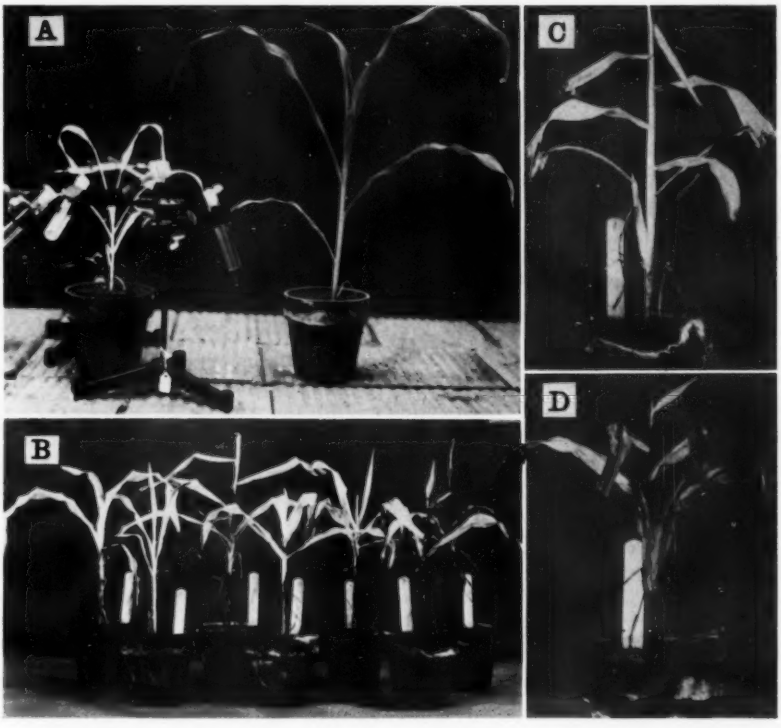

FIG. 3. *Albino maize plants. A. Half-grown plant showing feeding vials in place. Note shorter culm and smaller leaves as compared with adjacent green control. B. Typical appearance of mature plants irrespective of treatment. Third plant from left has small tassel. C. Mature plant with ear, lower left. D. Mature plant with ear, lower right, and anthocyanin in the culm and leaf tips.*

Unlike *Lemna*, *Spirodela* made some growth with 2.5%SH during the third week in darkness. With 0.5%SH,CPYA medium, both species multiplied at the same rate; with 2.5%SH,CPYA, *Spirodela* multiplied at a significantly higher rate than *Lemna* which was adversely affected by this sucrose concentration. This difference may be the result of *Spirodela's* apparent ability to utilize inorganic nitrate in the dark. As the dry weights show, *Spirodela* did not accumulate sucrose and convert it to starch to such an extent as *Lemna*.

Culture of Albinos

Burström (17) has reported that the leaves of wheat and other cereals require light and carbon dioxide for the reduction of nitrate although the roots can carry out the process in darkness. Failure of the nitrate reduction process in leaves lacking pigments may be a factor causing the growth of albinos fed sucrose to be slower and less vigorous than the growth of comparable green plants. Four lots of 10 albino maize seedlings were grown in soil for a period of 16 weeks. One lot received only sucrose, while the other three lots received in addition, corn seed diffusate, coconut milk, or growth factor supplement X containing nitrate, ammonium, and casein hydrolyzate. Sibling green plants alongside served as controls.

Albinos developed much more slowly than the green controls. Culm elongation was retarded; the leaves were short and narrow (Fig. 3A), full of starch, and often had pronounced red streaks of anthocyanin in the vicinity of the cut ends immersed in sucrose solution (Fig. 3D). When the experiment was concluded, the albinos had produced 13 to 15 leaves, the same number as the controls. One plant had a poorly developed tassel (Fig. 3B), and a few plants had small ears (Fig. 3C, D). The test solutions produced no significant increase in growth beyond that achieved with sucrose alone. From average measurements (Table XV), it is apparent that albinos had shorter, narrower culms, narrower leaves, and much less top and root weight than the green controls. The controls themselves were not especially vigorous specimens, having been grown in small pots with no direct sunlight.

TABLE XV

AVERAGE MEASUREMENTS FOR MAIZE PLANTS AT THE CONCLUSION OF 16 WEEKS GROWTH

Type	No. of plants	Culm height, cm.	Culm diameter, mm.	Leaf width, mm.	Plant dry weight, gm.	
					Top	Roots
Albino	30	9.8	4.1	16.3	1.2	0.1
Green	4	63.0	6.3	30.9	6.7	0.9

Spoehr (74) found that albino maize did not grow as well in a medium in which nitrate of the nutrient solution was replaced by ammonium. The effect of omitting nitrate altogether and supplying organic nitrogen compounds (supplement Y) in 10% sucrose through the leaves was next

investigated. Nutrient solutions deficient in potassium or both potassium and nitrate were also tested. Albino and green plants receiving complete Hoagland's nutrient served as controls. There were four plants per treatment. The experiment was terminated four weeks after leaf-feeding was begun. By this time, the three nutrient deficient treatments had caused one plant to die and the remainder to look sickly; they resembled one another in appearance, and had produced from four to six leaves. Both albino and green controls were in a healthy state, and had produced seven and eight leaves, respectively.

In a single experiment, albino barley seedlings fed 10% sucrose through the leaves failed to outlive the untreated controls to any significant extent.

Discussion and Conclusions

The successful culture of *Lemna minor* in darkness for 189 days, without significant decrease in frond multiplication rate (Table VI), provides one of the first examples of a plant with organized structure that has been grown heterotrophically for a prolonged period in a manner similar to many unicellular organisms. Sucrose, which is looked upon as one of the early products of photosynthesis, when added to the medium, was by itself inadequate to sustain growth. Sources of organic nitrogen and other factors, as furnished by casein hydrolyzate and yeast extract, were required. The related duckweed, *Spirodela oligorrhiza*, can probably be grown in darkness for indefinite periods of time equally well (Table XIV).

Lemna can utilize glucose, fructose, or sucrose as carbon sources, but in the presence of sucrose, acetate or respiratory acids are not beneficial. Amino acids or mixtures of amino acids, but not ammonium, urea, or nitrate are utilized as nitrogen sources. There is also an essential growth factor in yeast extract. *Spirodela* and albino maize both utilize sucrose and nitrate. *Spirodela* can also utilize a mixture of amino acids, but complex organic nitrogen sources fed to albino maize through the leaves have no growth promoting effect, perhaps because of poor absorption and transport.

These requirements are compared in Table XVI with the heterotrophic requirements that have been reported for other species of plants, plant organs, and tissues. The flagellate protozoa seem unable to utilize sugars but utilize acetate, butyrate, or other short-chain fatty acids as carbon sources. Green algae use monosaccharides and acetate, but use disaccharides with difficulty or not at all. The remaining species can grow with glucose, sucrose, or other sugars but not with lower fatty acids as a carbon source. The protozoa and green algae can utilize such respiratory acids as pyruvic, lactic, and succinic, but this may depend, as for *Prototheca* and *Chlorella* (3, 51), on the reaction of the medium being low enough (pH 3.8) to suppress ionization and permit cellular penetration. *Lycopersicon* root tips respond negatively and *Helianthus* and *Nicotiana* tissue cultures respond poorly to respiratory intermediates – perhaps because of poor penetration. Succinic acid promotes

TABLE XVI

CARBON AND NITROGEN SOURCES UTILIZED BY VARIOUS PLANTS, PLANT ORGANS, AND TISSUES WHEN GROWING HETEROTROPHICALLY

(Utilized +, poorly utilized ±, unutilized −, variable utilization +/−)

Species	Carbon source			Nitrogen source				Comments	References
	Sugars	Lower fatty acids	Respiratory intermediates	Nitrate	Ammonium	Amino acids, amides	Peptones		
Flagellate protozoa:									
*Astasia ocellata**	−	+	±	−	+	+	+	Ethyl alcohol, thiamine (+/−)	21, 22, 29, 30, 31, 39, 42, 43, 45, 46, 47, 50, 55, 56, 57, 60, 61
*Chilomonas paramaecium**	−	+	±	−	+	+	+		
Chlorogonium euchlorum	−	+	±	+	+	+	+		
Euglena gracilis	−	+	±	−	+	+	+		
*Polytoma uvella**	−	+	±	−	+	+	+		
*Polytomella caeca**	−	+	±	−	+	+	+		
Green algae:									
Chlorella vulgaris	+	+	+/−	+	+	+	+	Disaccharides (±)	1, 3, 6, 7, 16, 50, 51, 71
Chlorella pyrenoidosa	+	+	+/−	+	+	+	+	For *Prototheca*, thiamine (+)	
*Prototheca zopfii**	+	+	+/−	+	+	+	+		
Scenedesmus acutus	+	+	+/−	+	+	+	+		
Diatoms:									
Navicula minuscula	+	−	−	+	+	+	+	Inulin, starch (+)	50
Nitzschia Palea	+	−	−	+	+	+	+		
*Nitzschia putrida**	+	−	−	+	+	+	+		
Moss protonemata:									
Ceratodon purpureus	+	−		+	+	+	+	Strain differences, *Funaria*, *Leptobryum*	24, 58, 64
Funaria hygrometrica	+	−		+/−	+	+	+		
Leptobryum pyriforme	+	−		+/−	+/−	−	+		
Fern prothallia:									
Asplenium sp.	+			+					38
Seed plants:									
*Artemisia vulgaris**	+			+				For *Lemna*, *Spirodela*, *Zea*, vitamins, growth factors (−). For *Lemna*, *Scorzonera*, yeast extract (+)	27, 41, 63, 74, 81, 93 This investigation
Cattleya amethystoglossa	+			+**	+**				
Cymbidium Pangbourne	+			+**	+**				
Oncidium sp.	+			+**	+**				
Lemna minor	+	−	−	−	−	+	+		
Scorzonera sp.	+			+					
Spirodela oligorrhiza	+	−	−	±	−	+	+		
*Zea mays**	+			+	±		−		
Excised embryos:									
Cattleya spp.	+			+**	+**	+		For *Pisum*, ascorbic acid (+)	8, 14, 52, 53, 54, 69, 70, 75
Datura stramonium	+			+	+	+	+		
Pisum sativum	+			+					

* *These species lacked chlorophyll.*

** *Basal medium contained ammonium nitrate.*

TABLE XVI—*Concluded*

CARBON AND NITROGEN SOURCES UTILIZED BY VARIOUS PLANTS, PLANT ORGANS, AND TISSUES WHEN GROWING HETEROTROPHICALLY—*Concluded*

(Utilized +, poorly utilized ±, unutilized −, variable utilization +/−)

Species	Carbon source			Nitrogen source				Comments	References
	Sugars	Lower fatty acids	Respiratory intermediates	Nitrate	Ammonium	Amino acids, amides	Peptones		
Excised root tips:									
Acacia melanoxylon	+			+				According to species, yeast extract or thiamine, niacin, pyridoxine (+/−). For *Zea*, indolylacetic acid (+)	11, 12, 13, 15, 34, 48, 65, 66, 73, 80, 85, 87, 88, 89, 90, 91
Lycopersicon esculentum	+		−	+	−	+/−	+/−		
Pinus silvestris	+			+					
Pisum sativum	+			+	−†		+		
Zea mays hybrid	+			+		−			
Excised stem tips:									
Asparagus officinalis††	+		±	+	+	+			25, 44
Excised leaves:									
Brassica oleracea††	+			+			−	For *Brassica*, *Secale*, vitamins (−). For *Pisum*, purines (+), for *Secale* (−)	9, 10, 67, 68
Pisum sativum††	+			+					
Secale cereale††	+			+					
Tissue cultures:									
Daucus carota, root	+			+				According to species and nature of tissue, vitamins, growth factors (+/−)	26, 35, 36, 37, 62, 72, 86, 88, 89, 90, 91
Helianthus annuus, gall	+	−	±	+	+	+	+		
Nicotiana hybrid, callus	+	−	±	+					

† *Substituting ammonium nitrate for potassium nitrate failed to improve growth.*
†† *Limited growth only.*

growth of *Asparagus* stem tips, but only during the first transfer. None of the protozoa listed except *Chlorogonium* are able to utilize nitrate, although ammonium, specific amino acids or amides, and peptones are suitable nitrogen sources. Green algae and diatoms, as well as certain moss protonemata, excised embryos, and tissue cultures can use nitrate, ammonium, or numerous organic nitrogen sources. *Lycopersicon* root tips utilize nitrate, but ammonium is not utilized and the effects of amino acids, asparagine, and peptone are not clearly defined. *Pisum* root tips, also, may be unable to utilize ammonium. The remaining species, which have been investigated less completely, utilize nitrate.

Table XVI reveals many similarities in the carbon and nitrogen sources utilized for heterotrophic growth by plants and tissues and also a number of differences. Some of these differences may be the result of inadequate feeding techniques and culture media. For example, *Lemna* may have appeared

unable to utilize respiratory intermediates as a carbon source because sucrose was available simultaneously. Poor acid penetration at pH 4.8, as mentioned for *Chlorella* and *Prototheca*, is another possible explanation, although several protozoa are reported to utilize one or more of the acids, lactic, pyruvic, or succinic at pH 6.0 to 7.0 (46, 47, 55, 56, 57).

Whereas most green plants and tissues utilize nitrate in light and darkness (some growing equally well under both conditions), the strain of *Lemna* used in this investigation can do so only in light. This suggests that ordinarily two processes of nitrate assimilation exist, and that in *Lemna* the one that proceeds in darkness is blocked. Burström (17, 18) has concluded from experiments with wheat leaves that there are two such processes, dominated under normal circumstances by the one requiring light. He believes the dark assimilation to be of minor importance, and to be identical with carbon heterotrophic nitrate utilization which occurs in roots, bacteria, and fungi. It involves reduction to the level of ammonium and then assimilation into organic linkage. According to this scheme, *Euglena* and a strain of *Funaria* (24) which can utilize ammonium but not nitrate in the dark, and *Lemna* and a strain of *Leptobryum* (58) which can utilize neither (Table XVI) have blocks at different points. *Lycopersicon* root tips, however, have been reported to use nitrate but not ammonium (66) – contrary to the scheme. *Lemna* is unable to grow in light with ammonium as the sole nitrogen source (2), which is in accordance with Burström's conclusion that the light process does not involve ammonium. Differences in the manner of nitrate assimilation are perhaps the most important yet to be observed between the nutrition of green plants in light and in darkness.

The specificity of certain amino acids for growth of *Lemna* and the inhibitory or toxic effects of others (Table VII) is in agreement with similar observations made for a number of the protozoa, algae, and the other heterotrophs listed in Table XVI (1, 7, 22, 45, 56, 57). The same has been found for entire seed plants in light (5; 49, p. 57; 76; 77; 83), isolated embryos in light and darkness (69, 75), and tissue cultures in darkness (62). Within narrow concentration limits a number of amino acids can be utilized in place of inorganic nitrogen, but toxic and inhibitory effects are common and the response to individual acids varies with species, light conditions, and age (for embryos). Peptones and amino acid mixtures are relatively less toxic than free amino acids, perhaps because they are integrated intact into protein structures with little or no preliminary deamination for the synthesis of amino acids in short supply (82). From their observations with *Datura* embryos treated with various mixtures of amino acids, Sanders and Burkholder (69) have concluded that the effects of amino acids are not merely additive, but that there are interactions between them, and that a balance of amino acids is the controlling influence on growth and differentiation.

The effects of casein hydrolyzate on the growth of *Lemna* must be attributed to several amino acids whose effects are complementary. By eliminating or reducing the proportions of those acids that produce adverse effects and

increasing the proportions of those that stimulate growth, an amino acid supplement more effective than casein hydrolyzate might be devised.

The substances responsible for the inoculum effect probably represent more than just a carbon source such as sugar, since sucrose feeding and photosynthesis have additive rather than competitive effects on their accumulation (Tables XII and XIII). The decreased inoculum effect following accelerated growth in light in response to CPYAT feeding (Fig. 2) indicates that nitrogen metabolism is involved. Products of nitrate assimilation in light that accumulate are probably used for initial growth in darkness. Thereafter, the growth process becomes adapted to utilize to some extent the sucrose, casein hydrolyzate, and yeast extract supplied externally. It is perhaps significant that in light, 1%SH,CPYAT caused an increment in MR over that of the 2.5%SH control of 28 to 39 (Table X), which is very similar to the maximum steady-state multiplication rate attained in darkness. This is another indication that the heterotrophic process may be quite distinct from the autotrophic process of growth.

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NUCLEAR CONDITIONS IN NORMAL STEM TISSUE OF *VICIA FABA*[1]

By L. C. Coleman

Abstract

Previous cytological studies of plant tumors have been carried out on unsuitable material, and on the assumption that the normal tissues in which tumors are induced are exclusively diploid. The present study investigates the condition in normal stem cortex and pith of *Vicia faba*, in which tumors can be regularly induced through inoculation with pure cultures of *Agrobacterium rubi*. Mitoses were induced by treatment with indole-3-acetic acid, and it was found that a very considerable degree of polyploidy exists in both tissues. In the pith the percentage of polyploid mitoses increases with some regularity from the nodal to the mid-internodal region, where it may be as high as 50% of the total. In the cortex, the difference in this regard is less striking, but still exists. Both tetraploid and octoploid cells were found, the latter being less than 10% of the total. In both cases, there was usually close association of homologues in both pro- and metaphase, but in the cortex a considerable number of nuclei did not show this association. The pairing is at times accompanied by chiasma formation. These findings must be taken into account in studying the relation of polyploidy to tumor development.

Introduction

In a previous publication (3), I have described the induction and development of tumors on stems of *Vicia faba* as a result of inoculation with pure cultures of *Agrobacterium rubi*, the object being provision of suitable material for cytological study. Previous cytological studies of plant tumors have been on those caused by *Agrobacterium tumefaciens*, and in plants with comparatively large numbers of small chromosomes, little suited to cytological investigation. Two of the most extensive studies have been those of Winge (22), on the crown gall of the sugar beet, and Levine (16), on crown gall of beet and tobacco. These three plants suffer from the disadvantages mentioned above. On the other hand, *Vicia faba* has a small chromosome complement ($2n = 12$) of exceptionally large chromosomes, showing a considerable degree of morphological differentiation. Fig. 1 reproduces a photomicrograph of a metaphase nucleus. As will be seen, there is one long chromosome pair with an approximately median centromere and with a nucleolar construction cutting off what may be considered as a very large satellite. This is usually designated as the "M" chromosome. The other five pairs have a subterminal centromere and are about half the length of the "M" chromosome. They differ somewhat in length and could be distinguished from one another were that considered necessary, as, for instance, in the case of reciprocal translocations. Fig. 2 shows a very early anaphase from a diploid pith nucleus. Here the two "M" chromosomes have been marked by arrows at the point of the satellite attachment. Fig. 3 reproduces a metaphase plate from a root tip illustrating the difference in length of two of the short chromosomes.

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A reciprocal translocation has been induced between two chromosomes at the point marked by an arrow. This is the result of treatment of the root with a very dilute solution of the salt of a nitrogen mustard, methyl*bis*(β-chloroethyl)amine hydrochloride. The difference in length of the two chromosomes involved clearly shows that they are not a homologous pair.

Both Winge and Levine lay stress on the existence of polyploidy as a characteristic feature of tumor tissue. Winge goes so far as to suggest that the presence of diploid nuclei in tumors is due, not to the persistence of the diploid condition, but to the occurrence of somatic reduction from the tetraploid state. More recent cytological work on animal tumors, notably that of Biesele *et al.* (2), has shown that, while polyploidy and polyteny are more frequent in tumor than in normal tissue, they are not confined to the former.

Before any conclusion can be reached as to the presence of polyploidy as a characteristic feature of plant tumors, it is necessary first to consider the situation in normal tissue. Litardiere (18), as long ago as 1923, was able to establish the presence of tetraploid nuclei in the root meristem of *Spinacia oleracea*, while Langlet (14), found a similar condition also in the root meristem of spinach. Since the time of these authors, various investigators, including notably Lorz (19), Gentscheff and Gustafsson (6), and Berger (1), have shown that the presence of polyploid (usually tetraploid) nuclei is a common occurrence in the root periblem of *Spinacia* species and that these cells have arisen, not by the fusion of diploid nuclei, but by chromosome splitting without a succeeding mitotic separation. Lorz (20) has recently published a review of literature dealing with polyploidy and associated phenomena in plants, from which it can be seen that investigations have been largely confined to the root tissue of plants from widely separated families.

Materials

During and since the war, Geitler (5), Jähnl (12), and others have studied mitoses induced by wounding in certain above-ground plant tissues, such as the walls of fruit capsules and the tissues of succulent leaves. They found various degrees of polyploidy characteristic of certain cell layers in these organs. Other investigators, notably Huskins and Steinitz (9, 10), have studied nuclear division induced by various hormones, such as indoleacetic acid and α-naphthaleneacetic acid, in differentiated tissue of the roots of various plants, especially *Rhoeo discolor*. They have shown the existence of polyploid nuclei and have correlated this finding with the presence of an increased number of heterochromatic bodies (prechromosomes) in the corresponding resting (energic) nuclei.

It has been argued that the action of stimuli, such as wounding or the application of hormones, might induce the polyploidy that these authors have set out to study. Thus Levan (15) found that onion roots, left in an aqueous solution of α-naphthaleneacetic acid for four days, developed a swelling in the region immediately above the apical meristem. A microscropic study of the swollen region revealed tetraploid nuclei in mitosis.

From the fact that he found, both in prophase and metaphase, four chromatids united by one centromere, he concluded that the extra split had resulted from the application of the growth substance, and had not simply been revealed by it. Similarly, Dermen (4) reported polyploid cells in the tumorlike swellings on bean plant internodes induced by the application of naphthaleneacetic acid in lanolin and concluded that the polyploidy was induced by the treatment, and was not already existing in the differentiated cortical parenchyma. He also reported the formation of C-chromosomes similar to those resulting from colchicine action.

Levan's observations and drawings, if correct, certainly give strong support to the view that growth substances induce rather than reveal polyploidy. His findings were so different from those generally reported, and from my own which are being reported in this paper, that I felt it necessary to repeat his experiments. In duplicating his experiments on onion root tips, I obtained exactly the same swelling immediately above the apical meristem. When tissue from this swollen region was fixed in Carnoy (acetic alcohol), hydrolyzed, Feulgen-stained, and squashed on a slide, tetraploid mitoses were found mixed among diploid ones, as was reported by Levan. However, instead of finding, in these nuclei, groups of four chromatids united in one centromere, or a number of four-strand chromosomes, I found pairs of two-strand chromosomes, each individual pair with its own centromere. Fig. 4 reproduces a photomicrograph illustrating these findings, the position of centromeres being marked by arrows. Pressure has more or less separated the paired chromosomes, but there is not the slightest evidence of any breakage of a centromere by the pressure. I have, therefore, to conclude that Levan's observations were incorrect in this regard, and that the situation, as regards chromosome pairing, is precisely the same in these nuclei as has been described for the natural conditions in the root periblem of spinach and other plants.

Geitler and Huskins have advanced a number of strong reasons supporting the view that the stimulus supplied by wounding or the action of a growth substance does not affect the chromosome number in the nuclei of the tissue studied, but, through the induction of mitosis, reveals a condition which has already existed previously. The strongest evidence supporting this view is the fact that, when root tips are left in the hormone solution for from 24 hr. to four days, the numerous divisions in the lowest portion of the apical meristem reveal exclusively a diploid condition, polyploid divisions being confined to the region above. It is highly improbable that the induction of polyploidy, if such occurred, would be confined only to one of two regions lying so close together. It must be admitted that this is no certain proof. However, I shall produce later in this paper evidence against polyploidisation by growth substances, which I believe is stronger than any so far advanced.

In my study of nuclear conditions in stem tissue, I have confined my attention to those tissues which mainly furnish the foundation material for stem tumors. These are, of course, the cortex and the pith. The fact that the tumors grow right through the stem and protrude on the other side, is

evidence that the pith can take part in tumor formation. As a matter of fact, incipient pith tumors can be seen at a very early stage if an infected internode is split open.

Methods

To induce divisions in the cortex, I applied indole-3-acetic acid dissolved in lanolin (2 mgm. per gm.) to the stem, and then made fine needle pricks in the epidermis to allow the more ready entry of the hormone. To induce divisions in the pith, I injected an aqueous solution of indole-3-acetic acid (50 p.p.m.) into the center of the stem downward from the region of the node. If pressure is applied slowly and evenly, the solution will be distributed more or less evenly, throughout the internode, although there is a considerable variation in this regard. In each case, treatment was carried out in the youngest node visible and the internode immediately below it. The material was fixed and stained from 46 to 48 hr. after treatment, by which time a considerable number of mitoses had usually been induced. The treated section, including the upper node, and the internode immediately below, was cut from the stem. The cortical layer on the side opposite to the treatment (injection or smearing), was removed and the remaining material fixed in Carnoy (alcohol–acetic acid 3 : 1), washed in water, hydrolyzed in normal hydrochloric acid for 15 min. at 60° C., and left in leucobasic fuchsin for from one-half hour to 24 hr. for staining. After being washed thoroughly in sulphur dioxide water, the material to be studied (cortex or pith) was carefully separated from the remaining tissue, including vessels and fibers, and small pieces were squashed on the slide in 50% acetic acid. Most of the microscopic study was carried out on such temporary mounts, but permanent mounts were also made by leaving the slides overnight in dioxane, separating coverslip from slide, and mounting in either dioxane balsam, clarite, or euparal.

Results

If we consider first the examination of pith tissue, it was found that both diploid and polyploid cells were present. The preparations gave clear pictures of all stages, including quite early prophase. Figs. 5 and 6 reproduce photomicrographs of diploid and tetraploid prophases respectively. In the former, the complete diploid complement can be quite commonly made out, while the latter, with its larger number of chromosomes, is not so clear in this regard. However, there can be seen clearly the close association of homologues, each already split into two chromatids. They show the characteristic relationship and coiling already pictured by other authors for the prophase polyploid nuclei in root periblem. One interesting feature which, as far as I am aware, has not previously been reported, is the presence of chiasmata between paired homologues in the prophase. The only previous reference to anything similar is that by Levan in onion root, where he represents, in a drawing, what could be interpreted as a chiasma, or, as he himself states, almost equally well as the sticking together of two adjacent chromatids. In the cases found by me, there can be no doubt whatever that we are dealing

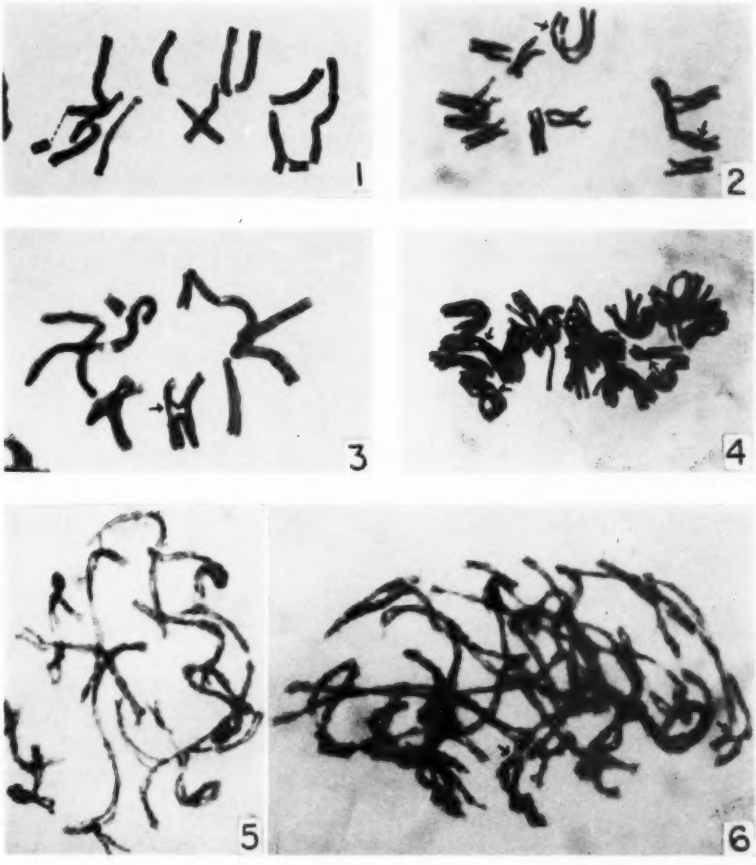
with true chiasmata. Fig. 7 shows this even more clearly than Fig. 6, the crossover being quite unmistakable. If any doubt remains in this regard, it must be dispelled by the identification of chiasmata in tetraploid metaphases. One such is illustrated in the photomicrograph reproduced in Fig. 10 at the point marked by an arrow. Fig. 9 shows a second tetraploid metaphase with the pairing of homologues clearly shown. Where these have been more or less separated by pressure they have been marked by arrows.

In this connection, it may be recalled that Huskins (8), in his paper on somatic reduction believed to be induced in root tips of *Allium* and *Rhoeo*, illustrated what are probably somewhat similar chiasmata. These are, however, at a much later stage of prophase than the ones illustrated by me. In my material, there is no evidence of chiasma formation being associated with somatic reduction, and there seems no possibility that it could have either genetic or ontogenetic significance. It is, however, of interest in revealing that the forces which induce breakage and chiasma formation are not confined to meiocytes.

Finally, a comparatively small number of octoploid nuclei have been observed at pro-, meta-, and anaphase. In octoploid prophases that could be analyzed there appears a striking difference from those previously described in root tissue. In the latter, octoploid nuclei appear to have arisen by stages, only one doubling having occurred in a single mitotic cycle. The result is that only pairs of homologues lie associated in pro- and metaphase. The pairs arising from a previous chromosome split have obviously been distributed during intervening mitoses. As can be seen in the accompanying Fig. 8, 12 groups each of four chromosome homologues are to be found associated throughout the prophase nucleus. This indicates the strong probability that two successive divisions of chromosomes have taken place in the resting phase following the last previous mitosis. A somewhat similar phenomenon is described and illustrated by Geitler for 16-ploid nuclei from fundamental tissue of the tuber of *Sauromatum guttatum*, a member of the *Araceae*. It also resembles a condition found by Painter and Reindorp (21) in the ovary nurse cells of *Drosophila melanogaster*.

It was found early in the examination of pith squashes that the ratio of polyploid (tetraploid and octoploid) cells to diploid ones varies greatly from preparation to preparation. This seemed to render any study of the relationship of tumor development to polyploidisation difficult, if not impossible. However, when a systematic study of pieces from different regions of the pith (nodal and internodal) was undertaken, it was found that the ratio was low at or near the node, and rose from there to the middle of the internode with some degree of regularity.

To obtain a more accurate estimate of this ratio, the pith cylinder was cut into pieces 2 mm. in length, starting at the point of injection about 2 mm. above the node and extending down to the middle of the internode immediately below. Each piece was squashed on a slide, and counts were made of the total number of diploid and polyploid mitotic figures. It was found that



FIGS. 1-6. *Magnified 800 diameters.*

FIG. 1. *Diploid metaphase from V. faba stem cortex. Note separation of end piece from one of long chromosomes due to pressure. The diploid number (12) can be easily counted.*

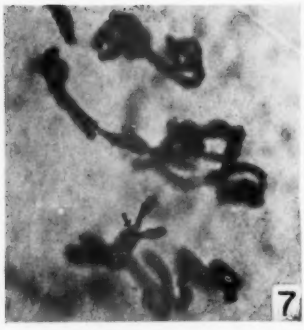
FIG. 2. *Diploid early anaphase in V. faba stem pith. The daughter chromosomes have just separated.*

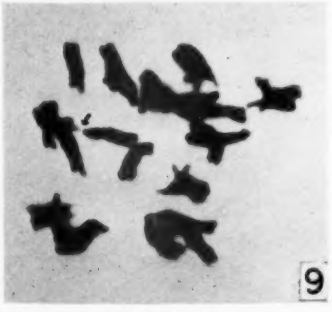
FIG. 3. *Diploid metaphase from V. faba root after treatment for 30 min. with dilute solution of a nitrogen mustard. At point marked by arrow there has been a reciprocal translocation between two nonhomologous short chromosomes of different length.*

FIG. 4. *Tetraploid metaphase from swollen region of root of Allium cepa after treatment with naphthalene-acetic acid. At points marked by arrows it can be seen that the paired homologues are not united at the centromere as claimed by Levan.*

FIG. 5. *Diploid prophase from stem cortex of V. faba. The diploid number (12) can be counted in this early stage.*

FIG. 6. *Tetraploid prophase from stem cortex of V. faba showing close association of homologues and chiasmata at points marked by arrows.*





FIGS. 7-11. *Magnified 800 diameters.* FIG. 12. *Magnified 400 diameters.*

FIG. 7. *Tetraploid prophase from stem cortex of V. faba showing chiasma between two chromatids of one pair of homologues at point marked by arrow.*

FIG. 8. *Octoploid prophase from stem pith of V. faba showing homologues arranged in groups of four.*

FIG. 9. *Tetraploid metaphase from stem cortex of V. faba showing association of homologues in pairs. Compare this with Fig. 1.*

FIG. 10. *Tetraploid metaphase from stem cortex of V. faba showing chiasma at arrow.*

FIG. 11. *Octoploid early anaphase from stem cortex showing loss of association of homologues.*

FIG. 12. *Tetraploid prophase from stem cortex of V. faba showing loss of association of homologues.*

the total number of mitoses tends to fall from a high at or near the node to a low near the mid-internode. At the same time, there is a rise in the polyploid mitoses, both in numbers and in percentage of the total, from a low at the node to a high near the mid-internode. These findings are illustrated in the accompanying Table I giving figures for the numbers of diploid and of poly-

TABLE I

RECORD OF MITOSES IN 2 MM. SECTIONS OF PITH OF *V. faba* FROM NODE TO MID-INTERNODE

2 mm. sections starting at node	Number of diploid mitoses	Number of polyploid mitoses	Total	Percentage of polyploid mitoses
1	820	10	830	1.2
2	583	19	602	3.2
3	451	26	477	5.5
4	275	87	362	24.0
5	250	142	392	36.2
6	221	179	400	44.8
7	106	98	204	48.5
8	138	146	284	51.4
9	198	180	378	47.6
10	248	163	411	39.7
Total	3290	1050	4340	

ploid mitoses, the totals, and the percentage of polyploid mitoses in 2 mm. sections of the pith. The first two sections embrace the node, while those from 3-10 are sections progressively farther from the node. As will be seen, the total number of mitoses is very high at the nodal region, while in the internodal region the total number is lowest, in sections 7 and 8, which are close to the mid-internode, a region in which the percentage of polyploid cells is highest. The low total number of mitoses is due, undoubtedly, to the smaller number of cells in this region. This, in turn, is due to the larger proportion of polyploid cells and the fact that in this region the breakdown of cells in the middle of the pith has already begun. This breakdown in older stem sections embraces both nodal and internodal regions.

The accompanying diagram, Fig. 13, reproduces graphically these findings. Curve A represents the total number of mitoses shown on the ordinate line as multiples of 10. Curve B gives the percentage of polyploid mitoses to the total. The ordinates therefore represent actual numbers for Curve A and percentages for Curve B. The abscissa line is marked to represent 2 mm. sections, the actual points on which the curves are constructed being placed at the middle of each section. The node may be considered as at about 0 on the abscissa, while the mid-internode lies between 12 and 16.

Though the curves for other similar pith cylinders showed considerable variation from the one given, the general picture remained the same.

The number of mitoses was always highest at the node. The percentage of polyploid mitoses was always lowest at the node and highest at the mid-internode.

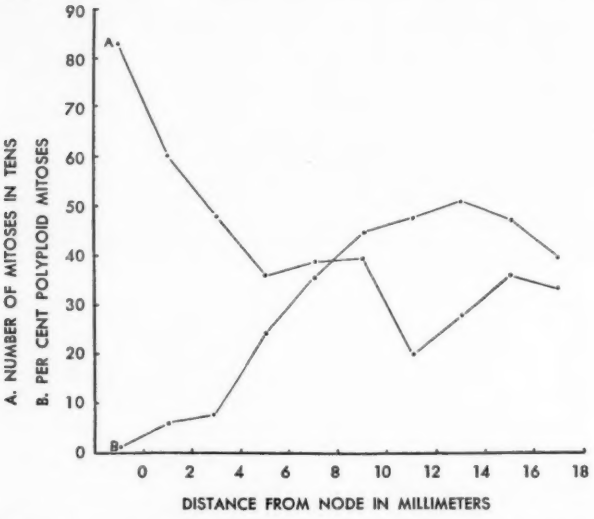


FIG. 13. *Diagram showing (A) total mitoses and (B) polyploid mitoses in 2 mm. sections of V. faba pith.*

I have not attempted here to separate octoploid from tetraploid in the polyploid nuclei. The percentage of octoploid nuclei was low, never being as much as 10% of the total polyploids. It is true that it was, at times, difficult to distinguish between a tetraploid and an octoploid prophase, but I feel sure that the error in this regard was small.

The above recorded data provide quite definite proof that exposure of differentiated cells to dilute aqueous solutions of a growth hormone such as indole-3-acetic acid does not lead to polyploidisation, but rather stimulates the cells to divide, thus revealing a polyploid condition already existing in the resting nuclei. Only in this way, can one account for the fact that, although the injection was made at or near the node, it was invariably in this region that the highest number of mitoses and the lowest number of polyploid mitoses was found. It seems to me inconceivable that a variation of this kind could be due to any difference between the pith cells at a node and those in the middle of the internode immediately below, or that there could be such a progressive increase as is shown by my counts.

An examination of cells of the stem cortex, after stimulation with indole-3-acetic acid in lanolin, gave a less striking difference in the percentage of polyploid cells at the node and those at the mid-internode, but a difference of the same general character was found here also. The percentage of polyploid

mitoses at the node was never more than 5%, while that at the midinternode varied between 15 and 20%. In the cortex, tetraploid and octoploid prophases quite commonly did not show the association of homologues which seems to be universal in the pith. Fig. 12 shows a well spread out prophase, where almost a complete count can be made in the photomicrograph. Fig. 11 shows an octoploid early anaphase. In neither of these photographs is there evidence of homologue association. This indicates that, in these cases, the differentiation of polyploid cells has taken place at least three cell generations before the final differentiation of permanent tissue has occurred. If this were not the case, there would certainly be evidence of homologue association in these cells. This lack of association made it more difficult to identify polyploid prophases.

In all cases examined, the number of mitoses found, whether diploid or polyploid, was small compared to the total number of nuclei in the preparations. This, of course, applies to mitoses in ordinary meristematic tissue when no special stimulus has been applied.

The identification of resting polyploid cells in differentiated tissues of *V. faba* cannot be made. Huskins and Steinitz (9) have attempted, in root tissue of *Rhoeo discolor*, to make an estimate of polyploidisation on the basis of the number of heterochromatic bodies in resting nuclei. This method, even in the case of *Rhoeo*, encounters the difficulty that heterochromatic bodies often fuse and no very clear-cut results can be obtained. In the case of *Vicia faba*, the number of heterochromatic bodies (chromocenters) is small and variable, so that any attempt to use them had to be abandoned. In the case of animals, where the nuclei of tissues such as that of the liver are commonly subspherical, it has been possible to establish a geometric progression in the volume of nuclei such as would be expected as a result of doubling chromosome numbers. Jacobj (11) was the first to establish such a ratio in the case of rat and mouse liver cells, and Biesele *et al.* have used the same method in the study of polyploidy in normal and cancer tumor cells of the mouse liver

In the case of differentiated parenchymatous tissue, such as *Vicia faba* pith and cortex, where there are commonly large central vacuoles, the nuclei are greatly flattened. Even in face view they rarely show a spherical outline. I attempted to obtain an estimate of nuclear size by making outline drawings with a camera lucida on uniform drawing paper, cutting these out with a fine pair of scissors and weighing the resulting pieces of paper. I used for the purpose nuclei chosen at random which had an approximately circular outline. The weights showed a very wide variation of as much as 1 to 4. However, a curve prepared from the data obtained showed no well marked peaks, so the method was abandoned. Any appraisal of the extent of polyploidisation in normal stem tissue of *Vicia faba*, as well as any estimate of the relation of polyploidisation to tumor development, must be based on observations of mitoses.

Discussion

Two points of general interest appear to arise from these observations. Geitler and Huskins and their associates have attempted to correlate polyploidy in somatic tissue of diploid species (polysomaty, polyteny, etc.) with processes of differentiation in ontogeny. My findings would seem to lend support to their views, for both stem cortex and stem pith show a marked difference between node and internode in the degree of polyploidy.

The second point has to do with the production of polyploids through stem decapitation with or without hormone application to the cut surface. Jorgenson (13), Lindstrom and Koos (17), Greenleaf (7), and others were able to obtain a proportion of polyploid shoots among those developing from the callus formed as a result of this treatment. Lindstrom and Koos reported finding binucleate cells in the callus, and the suggestion is that the treatment was responsible for the polyploidisation. From my findings, it seems much more probable that the wound and hormone stimuli led to the production of polyploid tissue from polyploid cells already existing in cortex and pith. In this connection it would be interesting to investigate whether a greater percentage of polyploid shoots will develop when the decapitation has been made in the internode than when it is made at the node.

Acknowledgment

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